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(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 300 Lakeside Drive, 2nd Floor, Oakland, CA 94612-3550 (US).

(72) Inventors: WHITE, David, L. ; 9082 Broadway Terrace, Oakland, CA 94611 (US). EASON, Robert, G. ; 1256 Page Street, San Francisco, CA 94617 (US).

(74) Agents: PETERS, Howard, M. et al.; Phillips, Moore, Lempio & Finley, 177 Post Street, Suite 800, San Francisco, CA 94108-4731 (US).

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(54) Title: AMINO ACID, ESTER AND/OR CATECHOL CONTRAST AGENTS FOR MRI

(57) Abstract

The present invention relates to the preparation of amino acid containing compounds which also have multiple carboxylic acid functional groups. Paramagnetic metal (II) or (III) ion chelate complexes are formed using these compounds for use as intravenous contrast agents to produce enhanced contrast magnetic resonance images of the heart, liver, biliary tree or upper small intestine. The mono and di-amino acids (and their carboxylic esters carboxylic amides, and catechols) of EDTA, DTPA, and the like are prepared. The paramagnetic metal (II) or (III) ion complexes are formed and produce T1-related contrast effects in MR images. The compounds and complexes also appear to have low toxicities and to be relatively rapidly and completely cleared from the tissue of a living mammal, e.g. a human being.

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AMINO ACID, ESTER AND/OR CATECHOL CONTRAST AGENTS FOR MRI

BACKGROUND OF THE INVENTION

Related Applications

This application is a Continuation-In-Part of U.S. Serial No. 744,470, filed August 12, 1991 which is a Continuation-In-Part of U.S. Serial No. 743,143, filed August 9, 1991.

Field of the Invention

The present invention relates to the preparation and use of amino acid and catechol containing hepatobiliary and cardiac contrast agents useful in magnetic resonance imaging. The contrast agents have multiple carboxyl groups to chelate a variety of metal (II) or (III) ions.

Description of Related Art

This invention relates to contrast agents for medical magnetic resonance imaging (MRI).

A contrast agent is an exogenous substance that either augments or suppresses the normal in vivo MRI signal, thereby yielding additional diagnostic information. The theory (1,2) and applications of various types of contrast agents has been described in the literature (1,2). The Arabic numbers in parentheses in this section refer to the articles cited in this section.

The applications of a given MRI contrast agent are determined by its distribution in vivo. The mechanisms controlling the initial biodistribution can be classed as physico-chemical, i.e., dependent only upon such properties as molecular size, charge, lipophilicity, surface properties, etc., or receptor-mediated--dependent upon the binding of a substrate to a specific receptor in or on cells. Different organs may handle the same contrast agent by different mechanisms. For example, the molecular size of the agent may result in its filtration by the kidneys (or confinement to the vascular space) while it is cleared

by receptor-mediated transport in the liver.

Contrast agents exhibiting a physico-chemical distribution mechanism include the gadolinium (III) complex of diethylenetriaminepentaacetic acid (Gd-DTPA), which distributes in plasma and extracellular fluid, and albumin-$(Gd\text{-}DTPA)_n$, which remains largely intravascular (1,2). The former is used to demonstrate blood-brain barrier lesions or to reveal renal anatomy and function (3), while the latter has been used experimentally to delineate the vasculature (4) and determine brain blood volume (5,6). Iron-dextran, although a colloid, has a sufficiently long plasma half-life (12 hr) to be used as an intravascular T2 contrast agent (7), as do some superparamagnetic iron oxide particle preparations (8,9).

Because of its role in the removal of exogenous compounds from general circulation, the liver is able to actively take up and concentrate soluble, as well as particulate, contrast agents. The pathways followed by solutes from plasma to bile have been reviewed (10-11) and are diagrammed in Figure 4. Passage into the hepatocyte across the cell membrane can take place by pinocytosis, passive diffusion, and/or by carrier-mediated systems that transport bile acids, bilirubin, organic anions, organic cations, neutral organic compounds, or inorganic ions. The substrate specificity of different carrier systems can partially overlap (e.g., organic anions and bile acids). The substrate may be metabolized intracellularly and/or conjugated with glucuronic acid or glutathione, for example. Finally, excretion into bile canaliculi again involves passage through a cell membrane. The mechanism of biliary excretion for a given compound may differ from that operative for its uptake.

The relative rates of metabolism, biliary elimination, and renal excretion determine the clearance of drugs and their metabolites from plasma and their persistence in any

one organ system. However, presently the factors that direct one compound to be excreted in the bile and another in the urine are not completely understood. Molecular weight, polarity, and molecular structure in relation to binding to plasma and transporter proteins are important. There appears to be a general molecular weight threshold, which is species-dependent (ca. 300 for rats and 600 for humans) below which urinary excretion dominates (10-11). Hydrophilic-lipophilic balance appears to play a critical role in biliary excretion (26-28). However, a priori prediction is not presently possible.

The liver has provided the first example of receptor-mediated localization of an MR contrast agent -- Fe-EHPG (EHPG is Ethylene-bis(hydroxyphenylglycine)) (12). Other iron (13-15), manganese (16-17), and gadolinium (18) chelates have since been described that have either potential for, or have demonstrated receptor-mediated hepatocyte uptake.

It has been reported by others that the anionic chelates Fe-EHPG, Fe-HBED (HBED = bis-(hydroxybenzyl) ethylenediaminediacetic acid), and Fe-PGDF (PGDF = N-3-(phenylglutaryl)desferrioxamine B) are transported in the liver by a system or systems inhibitable by BSP (bromosulfophthalein) (13,15).

The lipophilic chelate Gd-BOPTA ("benzyloxypropionic-tetraacetate," a derivative of DTPA) was shown to have significant biliary excretion (38.6% of injected dose in bile at 6 hr) (18). No information was reported on the mechanism of transport (e.g., passive diffusion or anionic transport) of this compound. Gd-BOPTA produced a larger signal enhancement (48%) in liver than Gd-DTPA (16%) in T1-weighted spin-echo images at 0.5 Tesla.

Additionally, other organs and tissues may posses receptors with affinity for certain classes of substrates, e.g., amino acids, peptides or catechol amines (19-24).

These receptors may also bind molecules that resemble the substrate, e.g., a derivative of an amino acid that is present in a peptide substrate (22) or an amide derivative of a naturally occurring catechol amine such as dopamine. The contrast agents of this invention may in part localize by such a mechanism. Furthermore, the localization of the catechol containing contrast agents of the present invention may depend in part on their respective reduction-oxidation properties.

To date, magnetic resonance imaging (MRI) has played a minor role in imaging of the liver and abdomen of a human being because of degradation of image quality by motion artifacts, and by the lack of suitable contrast agents. Recent technical advances in instrumentation (e.g., self-shielded gradient coils) and pulse sequences (e.g., echo-planar and turbo-flash techniques) promise to alleviate the motion-related problems of the torso and abdomen, and make contrast agent development all the more important for continued progress in abdominal MRI.

General background in the use of MRI contrast agents and of their preparation and purification are described, for example, in:

H. Gries et al., U.S. Patent No. 4,647,447;

R.B. Lauffer et al., U.S. Patents 4,899,755 and 4,880,008;

B.L. Engelstad et al., U.S. Patent 4,909,257;

D.L. White et al., U.S. Patent 4,999,445.

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2. S. M. Rocklage, et al. "Contrast Agents in Amgnetic resonance imaging." Chapter 14, In Magnetic Resonance Imaging, 2nd ed, Stark DD, Bradley WG, eds. St. Louis: C.V. Mosby Co. (1992).

3. G. Bydder, "Clinical applications of Gadolinium-

DTPA." In Magnetic Resonance Imaging. Stark DD, Bradley WG, eds. St. Louis: C.V. Mosby Co. (1988); 182-200 (Chap. 10).

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11. C.D. Klaassen et al., "Mechanisms of bile formation, hepatic uptake, and biliary excretion," Pharm. Rev. (1984); 36:1-67.

12. R.B. Lauffer et al., "Iron-EHPG as a hepatobiliary MR contrast agent: Initial imaging and

biodistribution studies, J. Computer Assist Tomograph, (1985); 9:431-438.

13. B. Hoener et al., "Evaluation of Fe-HBED and Fe-EHPG as magnetic resonance contrast agents for assessing hepatobiliary function, J. Magn. Reson Imaging, (1991); 1:357-362.

14. K.A. Muetterties et al., "Ferrioxamine B derivatives as hepatobiliary contrast agents for magnetic resonance imaging, Magn Reson Med (1991); Vol. 22, pp. 88 to 100.

15. B. Hoener et al., "Hepatic transport of the magnetic resonance imaging contrast agent Fe(III)-N-(3-Phenyl-glutaryl)desferrioxamine B. Magn Reson Med. (1990); 17:509-51.

16. D.L. White et al., "Clearance, excretion, and organ distribution of a new MRI contrast agent Manganese-Dipyridoxal-Diphosphate (Mn-DPDP). Abstract Book: Society of Magnetic Resonance in Medicine (1988) 1:531.

17. S.W. Young, "MRI measurement of hepatocyte toxicity using the new MRI contrast agent manganese dipyridoxal diphosphate, a manganese/pyrdoxal 5-phosphate chelate," Mag Reson Med. (1989); 10:1-13.

18. P. Pavone et al., "Comparison of Gd-BOPTA with Gd-DTPA in MRI imaging of rat liver," Radiology (1990); 176:61-64.

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24. B. J. Clark. "The role of dopamine in the periphery," in The Dopaminergic System, B. Halasz, et al., eds., Springer-Verlag, Berlin, 1985, p 27-39.

All references articles, patents, etc. cited in this application are incorporated herein by reference in their entirety.

It would be very useful to have organic chelate metal ion complexes which are specific for MRI imaging of the liver, the biliary tree, the upper small intestine, or the myocardial tissue. The present invention provides complexes and methods having these useful advantages.

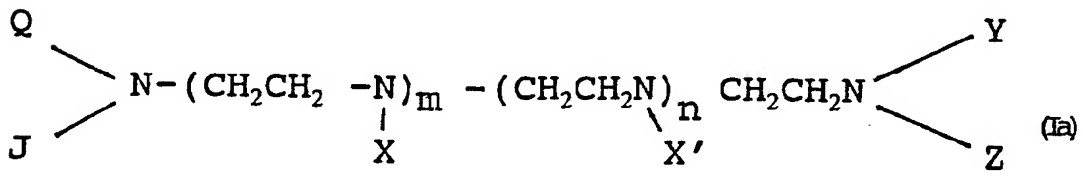
SUMMARY OF THE INVENTION

The present invention relates to a magnetic resonance imaging contrast agent, comprising the complex:

L-M

wherein M is a metal (II) or (III) ion independently selected from the group consisting of metals of atomic number 21 to 31, metals of atomic number 39 to 50, the lanthanide metals having an atomic number from 57 to 71, and metals of atomic number 72 to 82, and

L is a polydentate organic chelating moiety of structure Ia:



(Ia)

wherein

Q, J, X, X', Y and Z are each independently selected

from $-CH_2(C=O)-OH$, or $-CH_2(C=O)NHCH-(R)-(A)$;

wherein R in each of Q, J, X, X', Y and Z is independently selected from hydrogen or an organic structure comprising an alkyl, aromatic, substituted aromatic, alkylene aromatic, alkylene substituted aromatic, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group provided that at least one of Q, J, Y and Z is $-CH_2(C=O)-NHCH(R)-A$; and

A is independently selected from $-(C=O)OR^1$, $-(C=O)-N-(R^2)R^3$, or R^4 wherein R^4 is independently selected from $-CH_2$-aryl, $-CH_2$-substituted aryl, $-CH_2CH_2$-aryl, or $-CH_2CH_2$-substituted aryl, provided that when A is $-(C=O)OR^1$ and R^1 is hydrogen, then R is not hydrogen;

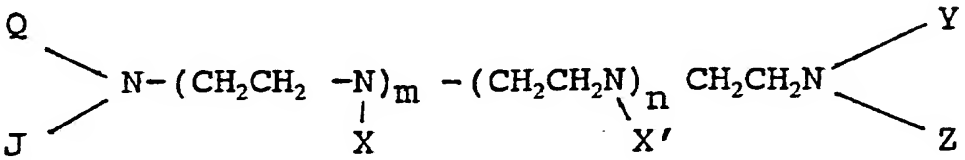
R^1, R^2 and R^3 when present in A in each of Q, J, X, X', Y and Z are independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl; and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1, or

the pharmaceutically acceptable salt(s) thereof.

In another aspect, the present invention relates to a polydentate organic chelating compound of structure I:



wherein

Q, J, X, X', Y and Z are each independently selected from $-CH_2(C=O)-OH$, or $-CH_2(C=O)NHCH-(R)-(A)$;

R in each of Q, J, X, X', Y and Z is independently selected from hydrogen or an organic structure comprising an alkyl, aromatic, substituted aromatic, alkylene aromatic, alkylene substituted aromatic, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group provided that at least one of Q, J, Y and Z is $-CH_2(C=O)-NHCH(R)-A$; and

A is independently selected from $-(C=O)OR^1$, $-(C=O)-N-(R^2)R^3$, or R^4 wherein R^4 is independently selected from $-CH_2$-aryl, $-CH_2$-substituted aryl, $-CH_2CH_2$-aryl, or $-CH_2CH_2$-substituted aryl provided that when A is $-(C=O)OR^1$ and R^1 is hydrogen then R is not hydrogen;

R^1, R^2 and R^3 when present in A in each of Q, J, X, X', Y and Z are independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl; and

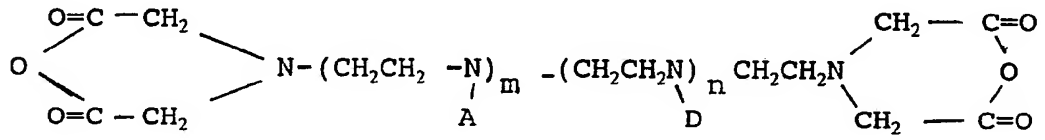
m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1, or

the pharmaceutically acceptable salt(s) thereof.

In another aspect, the present invention also relates to a method of preparing a chelate compound of structure (Ia), which method comprises:

(a) contacting a structure of the formula II:



wherein

A and D are each $-CH_2(C=O)-OH$,

with an amino acid, ester or $NH_2CH_2CH_2$ aryl or substituted aryl as defined herein:

$$H_2N-CH(R)-COOR^1$$

wherein R is an organic structure comprising an alkyl, aromatic or a heteroaromatic group, and

R^1 is selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl, and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1,

in an anhydrous polar aprotic solvent at between about 50 and 150° for between about 2 and 10 hr; and

(b) removing the solvent and recovering the compound of structure I.

These metal ion chelates produce T1 contrast effects in the heart, liver, biliary tree, and upper small

intestine. They demonstrate function, as well as anatomy. These contrast agents have low toxicities, and unlike iron from superparamagnetic particulates, the metal from these compounds should be rapidly and relatively completely cleared from the body. Therefore, these contrast agents are of substantial significance to useful abdominal MRI.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A, 1B, 1C and 1D are each a representation of the structures of the compounds BOPTA, BSP, DPDP and DTPA, respectively.

Figures 2A, 2B, 2C and 2D are each a representation of the structures of the chelates EDTA, EDTP, EHPG and HBED, respectively.

Figure 3 is a representation of a species of the general reaction to produce a bis amino acid substituted chelate.

Figure 4 is a cross-sectional representation of the cells, components and pathways found in the hepatobiliary region.

Figure 5A is a photograph of T-1 weighted magnetic resonance images of a rat at various times (indicated in minutes) after injection of the Gd-DTPA-(bisphenylalanine). Approximately 0.1 mmol/kg dose.

Figure 6A is a photograph of T-1 weighted magnetic resonance images at various times (indicated in min) obtained as in Figure 5A and Figure 5B for the Gd-DTPA-bis (phenylalanine ethyl ester).

Figs. 5B and 6B are photographic enlargements of the pre- and 0-min post injection images of Fig. 5A & 6A, resp.

Figure 7 is a photograph of the T-1 weighted magnetic resonance images of two mice side-by-side at various times (indicated in min) after simultaneous injection of Gd-DTPA-bisphenyl-alanine (bis acid) described in Example 8 below, at approximately 0.1 mmol/kg dose. The images are 2 mm thick slices in a coronal plane at the level of the heart.

The heart, liver and intestines are evident.

Figure 8 is a photograph of T-1 weighted magnetic resonance images as obtained for Figure 6 except that a different preparation of Gd-DTPA bis-(phenylalanine ethyl ester) was employed.

Figures 9-13 are each a graphic representation of MRI imaging in heart, lung, kidney, liver and skeletal muscle tissue, respectively, showing % enhancement versus time (min) for Gd(III)-DTPA-$(3HTA)_2$ and for Gd(III)-DTPA-$(DMPE)_2$.

Figure 14A is a photograph of T-1 weighted MRI images of a rat as obtained (as indicated in min) for Figure 5 using Gd(III)-DTPA-$(3HTA)_2$.

Figure 14B is a photograph of a second coronal plane at the level of the kidneys, as shown in Figure 14A.

Figure 15A is a photograph of T-1 weighted MRI images of a rat as obtained (as indicated in min) for Figure 5 using Gd(III)-DTPA-$(DMPE)_2$.

Figure 15B is a photograph of a second coronal plane at the level of the kidneys, as shown in Figure 15A.

Figures 16-20 are each a graphic representation of MRI imaging in heart, lung, kidney, liver and skeletal muscle tissue, respectively, showing % enhancement versus time (min) for Gd(III)-DTPA-$(L\text{-}PheOEt)_2$ and for Gd(III)-DTPA-$(D\text{-}PheOEt)_2$.

Figures 21A and 21B are each T-1 weighted MRI photographic images of a rat as obtained for Figures 14 and 15 using Gd(III)-DTPA-$(L\text{-}PheOEt)_2$ and for Gd(III)-DTPA-$(D\text{-}PheOEt)_2$. However, the dose level was 0.05 mmol/kg.

On Figures 9 to 13 and 16 to 20 solid vertical lines within the graph are shown ending in a horizontal line. The center box of this vertical line is the average for the observation at that point. The horizontal lines at either end of the vertical line are located at one standard derivation from the center value.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

Definitions

As used herein:

"Alkylene" refers to methylene, ethylene, propylene, and the like up to six carbon units.

"Amino acid" refers generally to the type of α-amino acids found in living subjects or mammals. However, synthetic α-amino acids which are not found in nature are also useful. Further these D- and L- amino acids as separate chiral isomers are independently useful. Mixtures of the D- and L- isomers are also contemplated in this invention.

"Metals of atomic number 21 to 29" refers to scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc and gallium respectively. Paramagnetic ions are especially preferred. Iron, manganese, nickel, chromium, cobalt are preferred.

"Metal (lanthanides) having an atomic number from 57 to 71" refers to lanthanide, cerium, praseodymium etc. to lutentium, respectively. Paramagnetic gadolinium (III) or dysprosium (III) are preferred.

The contrast agents of this invention localize in several organ systems, e.g., in the kidney, urinary tract, and urinary bladder; in the liver, biliary tree, and intestinal lumen; and in the myocardium. This localization results in increased MRI signal and image contrast. The resulting images show both improved anatomic detail and allow the functional state of certain organ systems, e.g., the urinary and biliary systems, to be ascertained.

This localization probably involves a combination of physico-chemical and receptor-based mechanisms. For example, binding to blood components results in enhancement of the blood pool and may contribute to heart enhancement. Localization in the liver may result from recognition and

transport by hepatocytes. Other mechanisms may also be involved. It may be possible to target other organs and tissues by selective modification of the structure of the metal chelate contrast agent.

Preparation of the amino acid-containing chelate (L) (having An Ester Group)

The following is a general description of the synthesis of the chelating ligand L. Specific descriptions are found in the Experimental Section.

In the synthesis of the compounds of structure I, the precursor can be DTPA-bis anhydride (or a similar structure, e.g. EDTA-bis anhydride) which contacted with an amino acid of the structure of the known natural or synthetic amino acids, e.g. D, L, or mixtures thereof. Generally, only one amino acid residue is added to one or more of the locations designated by Q, J, X, X', Y or Z, i.e., polypeptide bonds are usually not formed.

With the bis-anhydride, if a limited amount (e.g. 0.5 equivalent) of the amino acid is used, production of the mono amino acid derivative is favored. If two equivalents of amino acid is used, then the bis-amino acid derivative is produced. For DTPA or higher analogs of polycarboxylic acids, forcing conditions, such as using a coupling reagent and a large excess of the amino acid or protected amino acid may be required.

Any anhydrous dipolar aprotic solvent can be used for the synthesis. Dimethylformamide (DMF), dimethylacetamide, acetonitrile or the like are useful. DMF is preferred. The reaction mixture is heated at 70 to 100°C for between about 2-12 hr, preferably between 90 and 100°C for 4-5 hr, especially 6 hr.

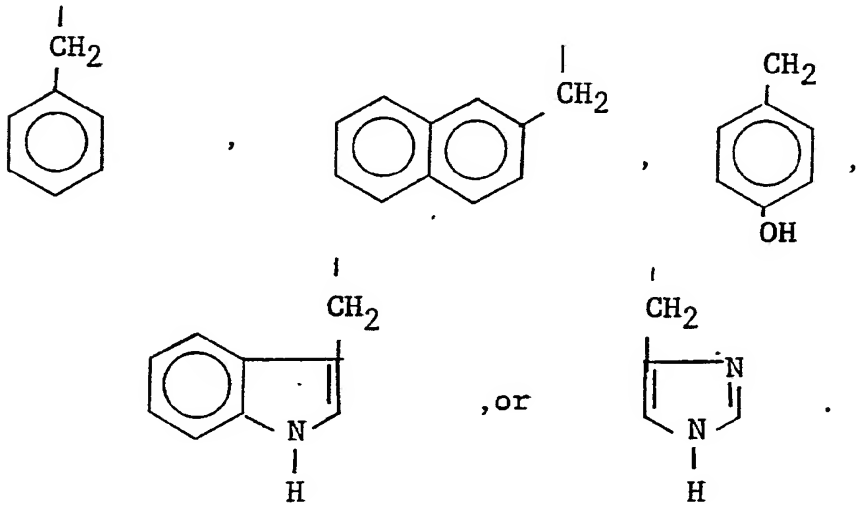
The reaction mixture is cooled and the solvent is removed using a conventional rotary evaporator or its equivalent. In one aspect, the present invention relates to a novel preparation of the compounds of structure I.

Preparation of the metal ion chelate complex (L-M)

The general description of the preparation of the chelate metal ion complex, L-M is conventional art. Refer to the references above.

Metal chelates are typically prepared by the reaction of a metal salt or oxide with the chelating ligand in a suitable aqueous or organic solvent in the appropriate stoichiometric ratio. Elevated temperatures are sometimes required. The pH of the reaction mixture is then adjusted with a base to obtain the corresponding chelate salt. Alternatively, acid can often be used to obtain the protonated chelate.

The R group preferred as independently selected from an aromatic group, an alkylene aromatic group, a substituted aromatic group or a heteroaromatic group. Especially preferred are the aryl aromatic groups shown below:



The R^1 group of the chelating ligand L in each of Q, J, X, X', Y and Z is independently selected from H (the acid), alkyl having from 1 to 7 carbon atoms (the mono, di, tri, etc. acid ester) cyclic groups such as cyclohexyl, phenyl, benzyl or 1- or 2-naphthyl.

Paramagnetic metal ions are preferred, especially iron (II) and (III) and gadolinium (III).

Amino Acid-Amide Structures

In another embodiment the present invention relates to specific structures wherein A is independently selected from $-(C=O)N(R^2)R^3$ wherein R^2 and R^3 are each independently selected from the group defined for R^1.

General Synthesis

The amides and related structures (free amide, mono substituted amide or disubstituted amide) are produced by starting with the appropriate amino acid amide (usually as the hydrochloride).

Some purification of the amino acid may be needed.

The amino acid amide is then contacted with the corresponding dianhydride as is described above for the amino acid ester. If a less than equivalent amount of amino acid amide is used and at high dilution in the solvent the mono amino acid amide is favored. If a stoichiometric excess of the amino acid amide is used the diamino acid amide structure is obtained.

Amide structures are also described in Examples 12 to 22. The amide structures are useful in MRI, because they have good contrast properties for specific tissue and have a longer useful half-life in a mammalian system.

SUBSTITUTED ALKYLENE ARYL DERIVATIVES

In another aspect the present invention relates to substituted alkylenearyl derivatives, (e.g. methylene catechols) of EDTA and DTPA-type structures.

The aryl and substituted aryl groups are defined as part of group R^4. When the $NH_2CH_2CH_2$-substituted aryl is

contacted with the bisanhydride as described for the corresponding amino acid ester or amide, the expected compound is obtained. When the substituents on the aryl group are hydroxyl, aqueous base should be avoided.

More specifically the present invention also concerns the preparation of a chelating ligand that bears one or more catecholamide groups, making a stable chelate of this ligand with a useful metal ion, and using the chelate for diagnostic imaging or spectroscopy. If the metal ion is paramagnetic, e.g., Gd(III) or Dy(III), the chelate can produce contrast enhancement in an MRI, or cause shifts, broadening, or other changes in a magnetic resonance spectrum.

These novel agents constitute an improvement over the prior art in that they tend to be localized in certain types of tissue by virtue of their resemblance to naturally occurring catecholamines and/or their redox and other physicochemical properties. In particular, two derivatives of dopamine (also 3-hydroxytyramine or "3-HTA"), DTPA-bis(3-hydroxytyramide), and DTPA-bis(3,4-dimethoxyphenethylamide), are useful. These ligands were reacted with GD(III) to produce the chelates, DTPA-$(3\text{-HTA})_2$ and GD-DTPA-$(3,4\text{-DMPE})_2$, respectively. These were used as contrast agents in the MRI of rats as described in the Examples. Both chelates demonstrated useful enhancement of heart, lungs, kidney and liver. However, the former selectively enhanced the heart.

Magnetic Resonance Imaging

In vivo magnetic resonance imaging of human organs and tissue is conventional and well established.

Figure 5 is a photograph of T-1 weighted magnetic resonance images of a rat obtained before, and at 0, 5, 10, 15, 25, 45, and 60 minutes after the injection of Gd-DTPA-bis(phenylalanine) at a dose of 0.1 mmol/kg body weight. The images are 60 mm x 60 mm x 3 mm thick slices in the

coronal plane. The region covered extends from just above the heart to somewhat below the liver. Enlargements of the pre- and 0-min post images are shown in Figure 5B. Imaging parameters are indicated along the left of the Figure and include the repetition time (3000000 microseconds), echo time (6000 microseconds, number of signal averages (4), and the image matrix size (128 x 256). The increase in signal intensity, particularly in the heart and liver, are readily apparent. Increase in signal intensity of the intestinal lumen is particularly apparent in the 25 min and later images, and suggests that contrast agent has been excreted into that organ.

Figure 6A and 6B are photographs of T-1 weighted magnetic resonance images obtained as described in Fig. 5A and 5B, except that Gd-DTPA-bis(phenylalanine ethyl ester) was used as the contrast agent. Note that this compound results in different apparent enhancement in the liver and heart as compared to that shown in Fig. 5A and 5B. These results suggest that the two compounds have significantly different biodistributions and pharmacokinetics.

Specific experiments are described in detail below in the Examples.

Administration of Contrast Agent

Any physician can determine the best mode of administration of the contrast agent. Generally, injection into a vein is used.

The contrast agents described herein are useful for the magnetic resonance imaging of the heart, liver, biliary tree, bladder and intestine of a subject, e.g. an animal, a mammal, especially a human being.

The following Examples are provided to further explain and describe the present invention. They are not to be construed to be limiting in any way.

EXAMPLE 1

PREPARATION OF DTPA-BIS(PHENYLGLYCINE)

In a 50-mL round-bottom flask equipped with a magnetic stirrer and a reflux condenser, and heated by an oil bath, was placed 1.10 g (3.08 mmol) of DTPA-bis(anhydride) (Aldrich Chemical Co.), 0.93 g (6.15 mmol) of d,l-α-phenylglycine (Fluka Chemical Co.), and 25 mL of dry dimethylformamide (Aldrich Chemical Co.). The reaction mixture was heated to 90-100°C and held within that temperature range for 6 hr. It was then allowed to cool to room temperature, and the solvent was removed using a rotary evaporator. The residue was washed by trituration with ether to yield 2 g of white solid of structure Ib (Figure 3).

EXAMPLE 2

PREPARATION OF THE Gd(III) COMPLEXES OF DTPA-BIS(PHENYLGLYCINE)

A solution of 2 mg (30 μmol) of DTPA-bis(phenylglycine) of Example 1 in 1 mL of water was treated with 14 mg (38 μmol) of $GdCl_3 \cdot 6H_2O$. The pH of the resulting was adjusted to 7.0 by addition of dilute sodium hydroxide solution. Insoluble $Gd(OH)_3$ was removed from the reaction mixture by filtration through a 0.22 μ filter. The T1 relaxation time of the resulting solution (1.3 mL volume) was 7 millisecond (ms)) at 0.25 Tesla and 37°C.

EXAMPLE 3

MAGNETIC RESONANCE IMAGING OF A RAT USING Gd-DTPA-BIS(PHENYLGLYCINE)

A 300 g male Sprague-Dawley rat was anesthetized with a intraperitoneal injection of a mixture of ketamine and diazepam, and a catheter was inserted into a lateral tail vein. The rat then was placed in a 5-cm inside diameter (i.d.) imaging coil in the bore of a 2-Tesla imager-spectrometer system (GE CSI; General Electric Co., Fremont, California). A T1-weighted spin-echo image of the animal's abdomen in the coronal plane was then obtained (TR 315 ms;

Te 15 ms; 128 x 256 image matrix; NEX = 4; 3 mm slice thickness). Next, 1.0 g of the Gd-DTPA-bis(phenylglycine) solution described in Example 2 was injected via the catheter. A series of post-injection images were obtained. The images displayed an initial small enhancement in the liver. As this enhancement decreased with time, increased intensity in the rat's small intestine then was observed, indicating hepatobiliary transport of the contrast agent. Intensity data are summarized below.

TABLE 1

IMAGE REGION-OF-INTEREST % ENHANCEMENT

Time (min post injection)	Liver	Small Intestine	Muscle
0-3	4	14	19
15-18	7	42	2
30-33	2	32	6

EXAMPLE 4

PREPARATION OF DTPA-BIS(L-PHENYALANINE ETHYL ESTER)

L-phenylalanine ethyl ester hydrochloride, 4.6 g (20 mmol; Sigma Chemical Co., St. Louis, MO), was dissolved in 15 mL of water and treated with 35 mL of saturated sodium bicarbonate solution. The resulting solution was extracted with four 10 mL portions of methylene chloride, and the organic extract was dried over anhydrous magnesium sulfate. The dried methylene chloride solution then was filtered to remove remaining drying agent, and the filtrate was concentrated to an oil using a rotary flash evaporator. This residue was further dried under high vacuum for several hours to yield 3.75 g of free base.

DTPA-bis(anhydride), 2.85 g (8.0 mmol), 10 mL of dimethylformamide (DMF), and 4.2 mL (24 mmol) of diisopropylethylamine (DIPEA) (Sigma Chemical Co., St. Louis, MO) were combined in a 50 mL round-bottom flask equipped with a

magnetic stirrer. The phenylalanine described above was dissolved in 10 mL of DMF, and the resulting solution added via syringe to the flask. The reaction mixture was warmed to 40°C, and then stirred for 13 hr at ambient temperature without external heating.

At the end of the 12 hr period, the reaction mixture was concentrated in vacuo to yield a viscous residue. This material was triturated with 100 mL of acetone, and the volatile components of the resulting mixture were removed in vacuo. The solid residue was recrystallized from a mixture of 125 mL of 60/40 water/ethanol. The white, crystalline product was washed with two 25-mL portions of cold ethanol, and the washed solid was dried in vacuo at 40°C for 1 hr to obtain 3.0 g (50% of theory).

Analytically pure product was obtained by dissolving 1 g of the above crystals in 75 mL of ethanol at 80-85°C, treating the resulting solution with decolorizing charcoal, removing the latter by filtration, and cooling the filtrate in an ice bath. Seed crystals were then added, and after 45 min, 0.6 g of recrystallized solid was isolated by filtration.

Anal: Calcd. for $C_{36}H_{49}N_5O_{12}$: C, 58.13; H, 6.64; and N, 9.42. Found: C, 57.75; H, 6.57; and N, 9.36.

EXAMPLE 5

PREPARATION OF DTPA-BIS(L-PHENYLALANINE BENZYL ESTER)

DTPA-bis(phenylalanine benzyl ester) was similarly prepared (according to Example 4) from L-phenylalanine benzyl ester p-toluene-sulfonic acid salt, 4.28 g (10 mmol; Sigma Chemical Co., St. Louis, MO). Ethyl acetate was used in place of ethanol for recrystallization. The yield was 2.6 g (75% of theory).

EXAMPLE 6

PREPARATION OF DTPA-BIS(L-PHENYLALANINE)

A solution of 1.23 g (1.42 mmol) of DTPA-bis(phenylalanine benzyl ester) in 15 mL of methanol was combined

with 0.1 g Pd/carbon catalyst (Aldrich Chemical Co, Milwaukee, WI) in a 25-mL round bottom flask. This mixture was treated with hydrogen gas at one atmosphere pressure for 6 hours. The reaction mixture was then filtered through a bed of diatomaceous earth filter aid. Volatile components were removed from the filtrate in vacuo. The yield was 0.94 g (97% of theory) of product, a somewhat hygroscopic white solid.

Anal: Calc'd. for $C_{32}H_{31}N_5O_{12}$*2HOH: C, 53.10 H, 6.27; and N, 9.68. Found: C, 53.13; H, 6.24; and N, 9.32.

When examined by HPLC, see description for Figure 8 below, the product was found to be about 10% bis acid, 45% bis ester and 45% mono acid mono ester. This is actually the contrast agent used for the Figure 6 MRI image.

EXAMPLE 7

PREPARATION OF THE GADOLINIUM (III) CHELATES OF BIS(PHENYLALANINE) AND ITS ESTERS

(a) A solution of 0.176 g (0.25 mmol) of DTPA-bis-(phenylalanine) in 4 mL of water was treated with 0.093 g of $GdCl_3$ (Aldrich Chemical Co., Milwaukee, WI). The pH of the resulting solution adjusted to 7.0 with aqueous sodium hydroxide solution. The volume was adjusted to 5.0 mL with water, and this solution was filtered through a 0.22 micron sterile filter into a sterile serum vial. The resulting 0.05 M solution is suitable for imaging in small animals.

The T1 relaxation time at 0.25 Tesla magnetic field strength and 37°C of a five-fold dilution of the above solution was 21 ms.

(b) The DTPA-bis(phenylalanine) mono and bis esters were prepared in a similar fashion.

EXAMPLE 8

MAGNETIC RESONANCE IMAGING OF A RAT USING Gd-DTPA-BIS(PHENYLALANINE)

A 300 g male Sprague-Dawley rat was anesthetized with a intraperitoneal injection of a mixture of ketamine and

diazepam, and a catheter was inserted into a lateral tail vein. The rat then was placed in a 5-cm inside diameter (i.d.) imaging coil in the bore of a 2-Tesla imager-spectrometer system (GE CSI; General Electric Co., Fremont, California). A T1-weighted spin-echo image of the animal's abdomen in the coronal plane was then obtained (TR 300 ms; Te 6 ms; 128 x 256 image matrix; NEX = 4; 3 mm slice thickness). Next, 0.6 g of the Gd-DTPA-bis(phenylalanine) solution described in Example 7 was injected via the catheter. A series of post-injection images were obtained. The images displayed an initial enhancement in the liver and heart. As this enhancement decreased somewhat with time, increased intensity in the rat's small intestine then was observed, indicating hepatobiliary transport of the contrast agent. Intensity data are summarized below. The intensity values show some fluctuations due to breathing motion and other small artifacts.

TABLE 2

IMAGE REGION-OF-INTEREST % ENHANCEMENT

Time (min post injection)	Liver	Heart	Muscle
0-3	51	42	14
5-8	60	27	3
15-18	47	25	12
25-38	50	22	12
45-48	34	9	10
60-63	33	12	2

EXAMPLE 9

MAGNETIC RESONANCE IMAGING OF A RAT USING Gd-DTPA-BIS(PHENYLALANINE ETHYL ESTER)

The imaging was carried out analogously to Example 8. Intensity data are summarized below.

TABLE 3

IMAGE REGION-OF-INTEREST % ENHANCEMENT

Time (min post injection)	Liver	Heart	Skeletal Muscle
0-3	70	86	50
5-8	109	46	30
15-18	113	66	31
25-38	99	48	26
45-48	71	49	16
60-63	44	25	16

EXAMPLE 10

(BIS PHE ACID ABOUT 100%) FIGURE 7

Two male BALB mice were imaged side-by-side in the same apparatus and using the same conditions as found in Example 8, except that the slice thickness was 2 mm. Over the illustrated time course from 0 min to 2.5 hr, the contrast agent can be seen to localize first in the liver (e.g. at 2 min), then in the gall bladder (at 90 min, for example), and then in the intestinal lumen (2-2.5 hr). It can also be seen in the urinary bladder.

EXAMPLE 11

COMPARATIVE MRI DATA IN MICE

Figure 8 is a photograph of T-1 weighted magnetic resonance images obtained as in Figure 6, except that a different preparation of Gd-DTPA-$(Phe\text{-}Et)_2$ was used.

When examined by HPLC (4.6 x 150 mm PRP-1) column; mobile phase -25 mmolar ammonia formate in water (Solvent A) and 50/50 (U/V) acetonitrile/water (Solvent B), programmed from 10% B to 95% B over 15 min, then holding at 95% B; flow rate 1 ml/min; U/V and/or radioisotope detector, the material used as a contrast agent in Figure 6 was found to have partially hydrolyzed to a mixture of Gd-DTPA- (ca 45%), Gd-DTPA-(Phe-Et) (Phe) (ca 45%), and Gd-

DTPA-$(Phe)_2$ (ca 10%).

Freshly prepared material, whose pH was carefully adjusted to neutrality, and which was stored in the cold, was determined to be about 90%. Gd-DTPA-$(Phe\text{-}Et)_2$ the remainder being mostly Gd-DTPA-(Phe)(Phe-Et).

The more pure preparation gave heart and liver enhancement (74 and 163% resp.) as shown in Figure 6 (84 and 53%, resp.). Thus the degree of liver enhancement was greater by a factor of about three.

These results suggest that esterified DTPA-amino acid chelates may be particularly advantageous for lower contrast enhancement.

Example 12

Preparation of DTPA-Bis(D-Phenylalanine Ethyl Ester)

DTPA-Bis(D-Phenylalanine Ethyl Ester) was prepared analogously to the corresponding L-isomer from D-phenylalanine ethyl ester and DTPA-bis(anhydride) (Example 4). The yield was 65%.

Anal. Calcd for $C_{36}H_{49}N_5O_{12}$: C, 58.13;H, 6.64; and N, 9.42. Found: C,57.87; H,6.55; N,9.48.

Example 13

Preparation of DTPA-Bis(Phenylalanine Methylamide)

A suspension of 2.07 g (10.43 mmol) of L-phenylalanine methyl amide hydrochloride in ethyl acetate (75 mL) was treated with a saturated aqueous solution of sodium carbonate (20 mL). The resulting solution was extracted with ethyl acetate (2 x 75 mL), and the combined organic extracts were dried over anhydrous sodium sulfate. The drying agent was removed by filtration, and the filtrate was concentrated to an oil using a rotary evaporator. This residue was further dried over P_2O_5 under high vacuum overnight to yield 1.72 g of the amine as a white solid.

A solution of the dried amine in anhydrous pyridine (15 mL) was combined with DTPA-bis(anhydride) (1.80 g, 5.04 mmol)under argon. The reaction mixture was heated at

reflux in an oil bath (95° C) for 60 min. The mixture was allowed to cool to room temperature (0.5 hr) and was concentrated in vacuo to yield a viscous residue. This material was dissolved in 100 mL of water, and the water then was evaporated in vacuo to yield a yellow oil. The oil was dissolved in a minimum amount of a solution of water in methanol (20% v/v) and treated with acetonitrile until a small amount of precipitate was observed. The precipitate was removed by filtration (0.45 μm membrane filter), and the filtrate was concentrated under reduced pressure. This procedure was repeated twice more, discarding the precipitate each time. Finally, the residue obtained by evaporation of the solvent was dissolved in a solution of water in methanol (10 mL, 20% v/v), and the desired product was precipitated by addition of a minimum amount of acetonitrile. The resulting white suspension was cooled in a freezer (-20° C) overnight, and the solvent then was removed by decantation. The product was dried under high vacuum (0.05 torr, 48 hr) over P_2O_5 and NaOH to afford 1.38 g (39%) of an analytically pure white solid.

Anal. Calcd. for $C_{34}H_{47}N_7O_{10}0.5\ H_2O$: C, 56.50; H, 6.69; N, 13.57. Found: C, 56.34; H, 6.61; N, 13.66.

Example 14

Preparation of DTPA-Bis(Phenylalanine Amide) and DTPA-Bis(Phenylalanine Dimethylamide)

The title compounds were prepared analogously to the dimethylamide compound (Example 13) from DTPA-bis(anhydride) and L-phenylalanine amide hydrochloride and L-phenylalanine dimethylamide in %20 and %59 yields, respectively.

Calcd for the amide $C_{32}H_{43}N_7O_{10}\cdot 2H_2O$: C,53.25; H,6.57; N,13.58. Found: C,53.43; H,6.30; N,13.59.

Calcd for the dimethylamide $C_{36}H_{51}N_7O_{10}$: C,56.90;H,7.03; and N,12.90. Found: C,56.82;H,6.74;N,12.76.

Example 15

Preparation of DTPA-bis(3-hydroxytyramide) ("DTPA-(3-HTA)$_2$")

DTPA-bis(anhydride) (3.57 g; 1.00 mmol; Aldrich Chemical Co., Milwaukee, WI) was suspended in 25 mL of anhydrous dimethylformamide (DMF; Aldrich) and treated with dopamine (3.78 g; 2.00 mmol; Fluka-USA, Ronkonkoma, NY) and di-isopropylethylamine (5.2 g; 4.0 mmol; Aldrich Chemical Co.) This mixture then was heated briefly to 100°C and sonicated for several minutes to dissolve the bulk of the solid. After stirring for 4-6 hr at 50-60°C, a deep yellow solution was produced. The reaction mixture then was allowed to cool to room temperature.

After stirring at ambient temperature overnight, the reaction mixture was concentrated on a rotary evaporator at 50°C to a volume of about 10 mL. The odor of di-isopropylethylamine was absent at this point. Water (25 mL) was added, and the resulting solution was washed twice with 20 mL portions of ethyl ether to remove the remaining DMF. The water then was removed *in vacuo* to yield a beige paste. This material was suspended in 10-20 mL of absolute ethanol and dried by azeotropic distillation of aqueous ethanol *in vacuo*. The crude product (7 g; 97% of theory) was a gritty, off-white, hygroscopic solid.

An analytical pure sample (1.76 g; 26% of theory) was isolated by preparative high-pressure liquid chromatography (HPLC) using a 4.6 x 150 mm Microsorb C-18 reversed phase column (Rainin Instrument Co., Emeryville CA). The mobile phase (1 mL/min flow rate) was a linear gradient from 5 to 50% acetonitrile in water over 12 min. An acidic pH was maintained by the presence of 0.1% v/v trifluoroacetic acid in both components of the mobile phase. A UV detector measuring absorbance at 276 nm was used. The retention time of the product was 13.5 min under these conditions. ^{1}H NMR spectrum: δ 6.75, m, 6H; δ 3.81-2.62, 26 H, aliphatic H, not further assigned.

Liquid secondary ion mass spectrum (LSIMS) $[M-H]^-$ = 662 (theory 662).

Example 16

Preparation of DTPA-bis(3,4-dimethoxyphenethylamide) ("DTPA-$(3,4\text{-DMPE})_2$")

Equimolar amounts of DTPA-bis(anhydride) and 3,4-dimethoxyphenethylamine (Aldrich Chemical Co.) were contacted as above in Example 15 to give crude product in ca 100% yield. This material was purified by preparative HPLC to produce 1.23 g (17%) of the title compound.

^{1}H NMR spectrum: δ 6.80, m, 6H; δ 3.82, s, 6H, CH_3O-; δ 3.80, s, 6H; CH_3O-; δ 3.45-2.76, 26 H, aliphatic H, not further assigned.

LSIMS mass spectrum: [M-H]- = 718 (theory 718).

Example 17

Preparation of Gd-DTPA-$(3\text{-HTA})_2$

Solutions of Gd-DTPA-$(3\text{-HTA})_2$ for imaging experiments were prepared by reacting DTPA-$(3\text{-HTA})_2$ in aqueous solution with a stoichiometric amount of $GdCl_3$ dissolved in water. After about 90% of the $GdCl_3$ had been added, the pH of the reaction mixture was adjusted to between 5 and 6 with aqueous NaOH solution. Xylenol orange indicator (1 drop of a 1 mg/mL aqueous solution) then was added, and $GdCl_3$ solution was added dropwise until the indicator changed from yellow to violet (at pH <6). The pH then was adjusted to between 7 and 8 with aqueous NaOH and, if necessary, aqueous Hcl solution. The reaction mixture was passed through a 0.22 μm sterile filter into a sterile serum vial. The final concentration ranged from 0.02 to 0.5 M, depending upon the initial concentrations of the reactants and the volumes of base and acid added for pH adjustment.

A sample of product for mass spectral analysis was obtained by HPLC (4.6 X 150 PRP-1 column; 1 mL/min flow rate; 5-45% over 15 min acetonitrile-in-water gradient).

The LSIMS $[M+H]^+$ parent ion peaks were observed from 815-824, with the maximum intensity at 819. The ratios of peak intensities were those predicted by theory $C_{30}H_{38}GdN_5O_{12}$.

Example 18

Preparation of Gd-DTPA-(3,4-DMPE)$_2$

This chelate was prepared analogously to Gd-DTPA-(3-HTA)$_2$ Example 17, above from DTPA-(3,4-DMPE)$_2$ and $GdCl_3$.

Example 19

In Vivo Magnetic Resonance Imaging Using Gd-DTPA-(3-HTA)$_2$ and Gd-DTPA-(3,4-DMPE)$_2$

Magnetic resonance imaging was carried out using a CSI 2-Tesla imager (GE, Inc., Fremont, CA) equipped with a 5-cm diameter distributed-capacitance imaging coil. A T1-weighted (TR 300/TE 6; NEX 4) spin-echo sequence was used. The image matrix was 128 X 256, the slice thickness was 3 mm, and the field-of view was 90 mm. Anterior (heart level) and posterior (kidney level) coronal image planes were used.

Sprague-Dawley rats (250-350 g; n = 4 for each contrast agent) were anesthetized with ketamine (90 mg/kg) and diazepam (10 mg/kg) and fitted with an intravenous catheter in a lateral tail vein. Anesthesia was maintained during imaging using pentobarbital delivered via an intraperitoneal catheter.

The anesthetized animal was placed in the imaging coil and secured with tape. The coil containing the animal then was placed in the magnet bore, and the magnetic field was shimmed. Pre-contrast images were obtained. The contrast agent (100 μmol/kg) then was injected via the tail-vein catheter, and additional images were obtained at various intervals for up to 90 min post injection.

Contrast agent enhancement was determined by measuring the mean signal intensity (SI) in operator-

designated regions of interest (ROI). These were normalized to the pre-injection value for each ROI according to the following formula:

$$\% \text{ Enhancement} = 100 \times (SI_{post} - SI_{pre})/SI_{pre}$$

The contrast enhancement (mean ± s.d., n = 4) as a function of time in heart, lung, kidney, liver, and skeletal muscle, Figures 9 - 13 respectively, for each of the contrast agents are illustrated, Gd-DTPA-$(3\text{-HTA})_2$ also tended to produce higher lung enhancement (186% ± 51% vs. 141% ± 4%). However, the differences between the effects produced by the two agents was smaller than in heart (cf. Figs. 9 and 10).

There was no significant difference in kidney enhancement (Fig. 11). Both agents produced ca. 175% enhancement 5 min after injection. The level of enhancement fell slowly over 70 min to about 100%.

About 50% enhancement was produced in the liver by both agents immediately post-injection (Fig.12). Additionally, the time course of enhancement was very similar for both agents, with the enhancement level falling to about 30% during the first 20 min post injection.

Skeletal muscle displayed peak enhancement of about 40% immediately post-injection. The enhancement-time curves for both agents were almost identical; each fell almost to pre-injection levels over 80 min (Fig.13).

Representative images using each agent are shown in Figures 14A and 14B and 15A and 15B as MRI photographic images.

Example 20

In Vivo Magnetic Resonance Imaging Using Gd-DTPA-$(L\text{-PheOEt})_2$ and Gd-DTPA-$(D\text{-PheOEt})_2$

The magnetic resonance imaging characteristics of the two contrast agents Gd-DTPA-$(L\text{-PheOEt})_2$ and Gd-DTPA-$(D\text{-PheOEt})_2$ were compared as in the previous Example using

groups of 4 and 5 animals, respectively. Figures 16 to 20 illustrate the contrast enhancement versus time behavior for each agent in heart, lung, kidney, liver and skeletal muscle, repectively.

Representative images using each agent are shown in Figures 21A and 21B and 22A and 22B as MRI photographic images.

Example 21

Hydrolysis of Gd-DTPA-(L-PheOEt)$_2$ and Gd-DTPA-(D-PheOEt)$_2$ in pH 7.4 Buffer and Rat Plasma

The rates of hydrolysis of the esters in rat plasma or pH 7.4 HEPES buffer were determined by addition of 10% by volume of Gd-153 radiolabeled 0.025 M chelate solution and incubation at 0 or 25°C. Aliquots were withdrawn at various time intervals and examined by HPLC [PRP-1 column; water-acetonitrile gradient; 25 Mm ammonium formate, pH 7 mobile phase].

The hydrolysis of either the LL- or DD-bis(ester) enantiomers to the corresponding mono(ester)-mono(acid) and thence to the bis(acid) in aqueous HEPES buffer at pH 7.4 and 25°C is very slow, with half-times for each step of the order of days.

However, the LL-bis(ester) is very rapidly hydrolyzed in rat plasma to the mono(acid)-mono(ester) (see below). The latter compound is much more resistant to hydrolysis of the remaining ester, with essentially no reaction being observed within 2 hr at 25°C.

In contrast, the DD-bis(ester) is resistant to even the first step of ester hydrolysis under these conditions (see below).

$t_{1/2}$ of Ester Hydrolysis in Rat Plasma

	@ 0°C	@ 25°C
Gd-DTPA-(L-PheOEt)$_2$	33 min	0.3 min
Gd-DTPA-(D-PheOEt)$_2$	None Detected	None Detected

The relative stability of the bis(esters) toward hydrolysis in aqueous solution versus plasma suggest that the plasma reaction is enzyme-catalyzed. Furthermore, mono(acid)-mono(ester) is evidently a much poorer substrate, as its rate of hydrolysis is much slower. This may be due to the change in net charge (from 0 to -1) of the chelate and/or to a change in conformation of the molecule due to coordination of the Gd by the free phenylalanine carboxylate group.

Changing the stereochemistry of the amino acid portion of the chelate to the unnatural D-enantiomer caused the rate of ester hydrolysis in plasma to greatly decrease.

Example 22

Determination of Relative Amounts of Urinary and Biliary Excretion

Male Sprague-Dawley rats were anesthetized with an intraperitoneal injection of mixture of ketamine (90 mg/kg) and diazepam (2 mg/kg), and fitted with a 23-guage cannula placed in a lateral tail vein. Next, a midline incision and a small lateral cut over the bile duct were made, and the bile duct was exposed. Two loose ties were placed proximally on the bile duct. A small nick was made distally, and the bile duct was cannylated with a 15-cm length of PE-10 polyethylene tubing, which was secured with the two ties.

A second piece of tubing was placed in the urinary bladder and secured with a purse-string suture. The flap of the abdominal wall was closed, and the incision was covered with gauze.

Heparinized (1 unit/mL) saline was infused at a rate of 0.075 mL/min via the iv catheter. After a 15 min stabilization period, the infusion was interrupted long enough to deliver a bolus dose (0.1 mmol/kg) of Gd-153 labeled contrast agent, and then resumed. Samples of

bile and urine were collected in tared tubes at regular intervals before and after injection of radiolabeled agent. The net weights of these samples were determined. The amount of Gd-153 present in each sample was determined by counting in a chamber gamma counter. The raw counts were corrected for background and normalized to the total amount of Gd-153 injected.

The Table below summarizes the results (cumulative 1 hr excretion; average of 3 animals) obtained for some of the agents described in the prior Examples:

One-Hour Cumulative Excretion

	Biliary	Urinary
Gd-DTPA-$(L\text{-}Phe)_2$	9.3±1.3	66.5±8.7
Gd-DTPA-$(L\text{-}PheOEt)_2$	30.5±7.4	46.9±8.0
Gd-DTPA-$(D\text{-}PheOEt)_2$	51.3±5.1	39.2±5.5
Gd-DTPA-$(L\text{-}PheNHCH_3)_2$	3.5±0.4	70.9±6.5

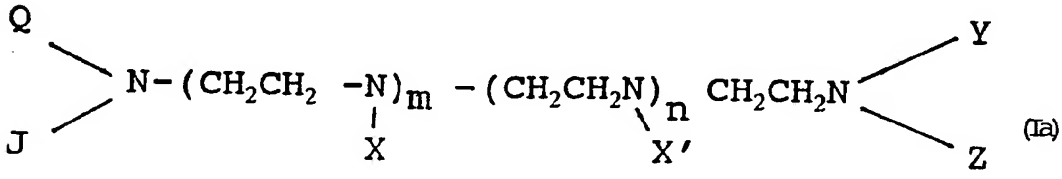
While only a few embodiments of the invention have been shown and described herein, it will become apparent to those skilled in the art that various modifications and changes can be made in the amino acid containing hepatobiliary or cardiac contrast agents or their use in magnetic resonance imaging of the torso or abdomen of a mammal without departing from the spirit and scope of the present invention. R or R^1 groups of the ligand L optionally comprise an aromatic or heteroaromatic moiety. All such modifications and changes coming within the scope of the appended claims are intended to be carried out thereby.

WE CLAIM:

1. A magnetic resonance imaging contrast agent, comprising the complex:

L-M

wherein M is a metal (II) or (III) ion independently selected from the group consisting of metals of atomic number 21 to 31, the lanthanide metals having an atomic number from 57 to 82, and L is a polydentate organic chelating moiety of structure Ia:



(Ia)

wherein

Q, J, Y and Z and X and X' when present are each independently selected from $-CH_2(C=O)-OH$, or $-CH_2(C=O)NHCH-(R)-(A)$;

wherein R in each of Q, J, X, X', Y and Z is independently selected from hydrogen or an organic structure comprising an alkyl, aryl, substituted aryl, alkylene aryl, alkylene substituted aryl, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group provided that at least one of Q, J, Y and Z is $-CH_2(C=O)-NHCH(R)-A$; and

A is independently selected from $-(C=O)OR^1$, $-(C=O)-N-(R^2)R^3$, or R^4 wherein R^4 is independently selected from $-CH_2$-aryl, $-CH_2$-substituted aryl, $-CH_2CH_2$-aryl, or $-CH_2CH_2$-substituted aryl, provided that when A is $-(C=O)OR^1$ and R^1 is hydrogen then R is not hydrogen;

R^1, R^2 and R^3 when present in A in each of Q, J, X, X', Y and Z are independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl; and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1, or

the pharmaceutically acceptable salt(s) thereof.

2. The contrast agent of Claim 1 wherein the α-amino acid(s) when present are selected from the D or L configuration or mixtures thereof.

3. The contrast agent of Claim 2 wherein at least one of J or Z is $-CH_2(C=O)NHCH-(R)-(A)$ and A is $-(C=O)-N(R^2)-R^3$, and R^2 and R^3 are independently selected from H or alkyl having 1 to 6 carbon atoms.

4. The contrast agent of Claim 3 wherein Q and Y are $-CH_2(C=O)-OH$ and X, X^1 when present are $-CH_2(C=O)-OH$ and m is 0 or 1 and n is 0.

5. The contrast agent of Claim 4 wherein one of R^2 or R^3 is hydrogen.

6. The contrast agent of Claim 5 wherein both J and Z are each $-CH_2(C=O)NH(CH)-R(A)$.

7. The contrast agent of Claim 4 wherein R^2 and R^3 are each alkyl.

8. The contrast agent of Claim 7 wherein J and Z are each $-CH_2(C=O)NHCH-(R)-(A)$.

9. The contrast agent of Claim 1 with the priviso that at least one R or A comprises an aryl, substituted aryl, alkylenearyl, alkylene substituted aryl, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group.

10. The contrast agent of Claim 1 wherein Q, X, X′ and Y are each $-CH_2(C=O)-OH$, and at least one of J and Z are
$-CH_2(C=O)NH\ CH(R)-A$ wherein A is $-CH_2$-aryl, $-CH_2$-substituted aryl, $-CH_2CH_2$-aryl, or $-CH_2CH_2$-substituted aryl, wherein aryl is selected from phenyl or naphthyl, and substituted aryl is substituted with 1 to 3 groups independently selected from halogen, alkyl having 1 to 7 carbon atoms, hydroxy, alkoxy having 1 to 7 carbon atoms, nitro, nitroso, amino or trifluoromethyl.

11. The contrast agent of Claim 10 wherein A is -

CH_2- substituted aryl wherein aryl is phenyl and is substituted with 1 to 3 alkoxy or hydroxy groups.

12. The contrast agent of Claim 11 wherein m is 0 or 1, n is 0 or 1; Q, X, X^1 and Y when present are each $-CH_2(C=O)-OH$, and at least one of J and Z are $-CH_2(C=O)NH-CH(R)-A$, wherein R is hydrogen and A is $-CH_2$-substituted aryl and is substituted with two alkoxy groups.

13. The contrast agent of Claim 12 wherein J and Z are $-CH_2(C=O)NH(R)-A$.

14. The contrast agent of Claim 11 wherein A is $-CH_2$- substituted aryl wherein aryl is phenyl and is substituted with two methoxy groups.

15. The contrast agent of Claim 12 wherein m is 0 or 1, n is 0 or 1; Q, X, X^1 and Y when present are each $-CH_2(C=O)-OH$, and at least one of J and Z are $-CH_2(C=O)NH-CH(R)-A$, wherein R is hydrogen and A is $-CH_2$-aryl and is substituted with two hydroxy groups.

16. The contrast agent of Claim 15 wherein J and Z are each $-CH_2(C=O)NH(R)-A$.

17. The contrast agent of Claim 11 wherein m is 0 or 1, n is 0 or 1; Q, X, X^1 and Y when present are each $-CH_2(C=O)-OH$, and at least one of J and Z are $-CH_2(C=O)NH-CH(R)-A$, wherein R is hydrogen and A is $-CH_2$-aryl, aryl is phenyl and is substituted with two hydroxy groups at the 3 and 4 positions of the ring, or with two methoxy groups at the 3 and 4 positions of the ring.

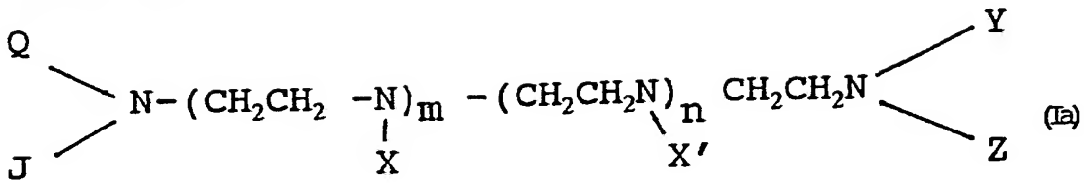
18. A magnetic resonance imaging contrast agent, comprising the complex:

L-M

wherein M is a metal (II) or (III) ion independently selected from the group consisting of metals of atomic number 21 to 31, metals of atomic number 39 to 50, the lanthanide metals having an atomic number from 57 to 71, and metals of atomic number 72 to 82, and

L is a polydentate organic chelating moiety of str-

ucture Ia:



(Ia)

wherein

Q, J, X, X′, Y and Z are each independently selected from $-CH_2(C=O)-OH$, or $-CH_2(C=O)NHCH-(R)-A$, wherein A is $COOR^I$; R in each of Q, J, X, X′, Y and Z is independently selected from hydrogen or an organic structure comprising an alkyl, aryl, alkylene aryl, alkylene substituted aryl, heteroaromatic, alkylene heteroaromatic group, substituted heteroaromatic, or alkylene-substituted heteroaromatic, provided that at least one of Q, J, Y and Z is $-CH_2(C=O)-NHCH(R)COOR^I$ provided that when R1 is hydrogen, then R is not hydrogen,

R^I in each of Q, J, X, X′, Y and Z is independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl; and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1, or

the pharmaceutically acceptable salt(s) thereof.

19. The contrast agent of Claim 18 wherein the α-amino acid(s) is selected from the D or L configuration or a mixture thereof.

20. The contrast agent of Claim 19 wherein M is a paramagnetic metal ion (II) or (III).

21. The contrast agent of Claim 20 wherein Q and Y are each $-CH_2(C=O)NHCH(R)COOR^I$,

J, X, X^I, and Z are each $-CH_2(C=O)OH$, and

m and n are each 0.

22. The contrast agent of Claim 21 wherein R is $-CH_2$-phenyl, and R^I is hydrogen.

23. The contrast agent of Claim 18 wherein M is a (III) metal ion, and

Q and Y are each $-CH_2-(C=O)NHCH(R)-COOH$ wherein R is selected from benzyl, p-hydroxyphenylmethyl, 2-methyl-naphthyl, or 3-methylindolyl;

J and Z are each $-CH_2(C=O)-OH$, m is 0 or 1, and X is $-CH_2(C=O)OH$, and n is 0.

24. The contrast agent of Claim 23 wherein the metal ion is selected from chromium (III), iron (III), cobalt (III), gadolinium (III) or manganese (II) or (III).

25. The contrast agent L-M of Claim 18 selected from the group of compounds of structure I consisting of the following groups:

M is independently selected from iron (III), chromium (III), manganese (II), dysprosium (III) or gadolinium (III);

Q is $-CH_2-(C=O)NHCH(R)-COOH$, R is $-CH_2$-phenyl, J, Y and Z are each $-CH_2(C=O)OH$, and m and n are each 0;

Q is $-CH_2-(C=O)NHCH(R)-COOH$, R is $-CH_2$-phenyl, J, X, Y and Z are each $-CH_2(C=O)OH$, and m is 1 and n is 0;

Q is $-CH_2-(C=O)NHCH(R)-COOH$, R is $-CH_2$-phenyl, J, X, X', Y and Z are each $-CH_2(C=O)OH$, and m and n are each 1;

Q and Y are each $-CH_2-(C=O)NH-CH-(R)-COOH$, R is $-CH_2$-phenyl, J and Z are each $-CH_2COOH$, and m and n are each 0; and

Q and Y are each $-CH_2(C=O)-NH-CH-(R)-COOH$, R is $-CH_2$-phenyl, J, X and Z are each $-CH_2COOH$, and m is 1 and n is 0.

26. The contrast agent of Claim 18 with the priviso that at least one R or one A comprises an aryl, substituted aryl, alkylenearyl, alkylene substituted aryl, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group.

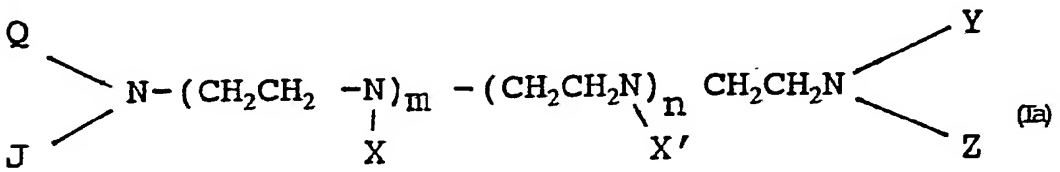
27. A composition useful as a contrast agent in magnetic resonance imaging of the tissue of a living

subject which composition comprises:

L-M

wherein M is a metal (II) or (III) ion independently selected from the group consisting of metals of atomic number 21 to 31, metals of atomic number 39 to 50, the lanthanide metals having an atomic number from 57 to 82, and metals of atomic number 72 to 82, and

L is a polydentate organic chelating moiety of structure Ia:



(Ia)

wherein

Q, J, Y and Z and X and X^1 when present are each independently selected from $-CH_2(C=O)-OH$, or $-CH_2(C=O)NH-CH-(R)-(A)$;

wherein R in each of Q, J, X, X', Y and Z is independently selected from hydrogen or an organic structure comprising an alkyl, aryl, substituted aryl, alkylene aryl, alkylene substituted aryl, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group provided that at least one of Q, J, Y and Z is $-CH_2(C=O)-NHCH(R)-A$;

A is independently selected from $-(C=O)OR^1$, $-(C=O)-N-(R^2)R^3$, or R^4 wherein R^4 is independently selected from $-CH_2$-aryl, $-CH_2$-substituted aryl, $-CH_2CH_2$-aryl, or $-CH_2CH_2$-substituted aryl, provided that when A is $-(C=O)OR^1$ and R^1 is hydrogen then R is not hydrogen;

R^1, R^2 and R^3 when present in A in each of Q, J, X, X', Y and Z are independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl; and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1, or

the pharmaceutically acceptable salt(s) thereof.

28. The composition of Claim 27 wherein Q and Y are each $-CH_2(C=O)NHCH(R)COOR^1$,

J, and Z are each $-CH_2(C=O)OH$, and

m and n are each 0.

29. The composition of Claim 28 wherein R is $-CH_2$-phenyl, and R^1 is hydrogen.

30. The composition of Claim 30 wherein M is a (III) metal ion,

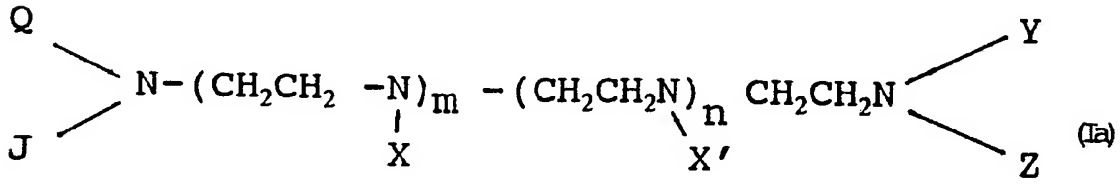
Q and Y are each $-CH_2-(C=O)NHCH(R)-COOH$ wherein R is selected from benzyl, p-hydroxyphenylmethyl, 2-methyl-naphthyl, or 3-methylindolyl.

J and Z are each $-CH_2(C=O)-OH$, m is 0 or 1, and X is $-CH_2(C=O)OH$ and n is 0.

31. The contrast agent of Claim 27 wherein the metal ion is selected from iron (III), cobalt (III), chromium (III), gadolinium (III) or manganese (II) or (III).

32. The composition of Claim 27 useful as an injectable contrast agent of a concentration of between about 0.5 and 5000 micromol/kilogram of body weight of a human being.

33. A polydentate organic chelating compound of structure Ia:



(Ia)

wherein

Q, J, Y and Z and X and X^1 when present are each independently selected from $-CH_2(C=O)-OH$, or $-CH_2(C=O)NHCH-(R)-(A)$;

wherein R in each of Q, J, X, X', Y and Z is independently selected from hydrogen or an organic structure comprising an alkyl, aryl, substituted aryl, alkylene aryl, alkylene substituted aryl, heteroaromatic, substi-

tuted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group provided that at least one of Q, J, Y and Z is $-CH_2(C=O)-NHCH(R)-A$; and

A is independently selected from $-(C=O)OR^1$, $-(C=O)-N-(R^2)R^3$, or R^4 wherein R^4 is independently selected from $-CH_2$-aryl, $-CH_2$-substituted aryl, $-CH_2CH_2$-aryl, or $-CH_2CH_2$-substituted aryl, provided that when A is $-(C=O)OR^1$ and R^1 is hydrogen then R is not hydrogen;

R^1, R^2 and R^3 when present in A in each of Q, J, X, X', Y and Z are independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl; and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1, or

the pharmaceutically acceptable salt(s) thereof.

34. The contrast agent of Claim 33 wherein the α-amino acid(s) when present are selected from the D or L configuration or mixtures thereof.

35. The contrast agent of Claim 33 with the priviso that at least one R or A comprises an aryl, substituted aryl, alkylenearyl, alkylene substituted aryl, heteroaromatic, substituted heteroaromatic, alkylene heteroaromatic, or alkylene substituted heteroaromatic group.

36. A method of examining human tissue in a diagnostic manner, which method comprises:

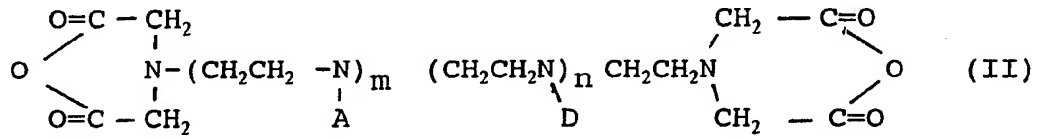
(a) injecting a human being with a contrast agent of Claim 1 in a dose amount having a concentration of the compound of structure Ia of between about 0.5 and 5000 micromol/kg of body weight in the human being;

(b) placing the human being in a magnetic field and irradiating the torso and abdomen of the subject of step (a) with radio-frequency energy such that nuclear magnetic resonance can be detected; and

(c) analyzing the imaging nuclear magnetic resonance signals obtained.

37. A method of preparing a chelate compound of Claim 33 of structure (Ia) which method comprises:

(a) contacting a structure of the formula II:



(II)

wherein

A and D are $CH_2(C=O)-OH$,

with an amino acid or ester of the structure:

$H_2N-CH(R)-COOR^1$ or amide of the structure $H_2N-CH(R)CONR^2R^3$ or a derivative of the structure $NH_2CH_2-CH_2$-substituted aryl as defined herein above

wherein R is an organic structure comprising an alkyl, aryl, alkylene aryl, a heteroaromatic or alkylene heteroaromatic group, and

R^2 and R^3 are independently selected from hydrogen, alkyl having from 1-7 carbon atoms, phenyl or benzyl, and

m is selected from 0, 1, 2 or 3, and

n is selected from 0 or 1,

in an anhydrous dipolar aprotic solvent at between about 50 and 150° for between about 2 and 10 hr; and

(b) removing the solvent and recovering the compound of structure Ia.

38. The method of Claim 37 wherein the dipolar aprotic solvent is independently selected from dimethylformamide, diethylformamide, hexamethylphosphoramide, dimethylsulfoxide or mixtures thereof; and

the heating temperature is between about 90 and 100°C and the time is between about 4 to 7 hr.

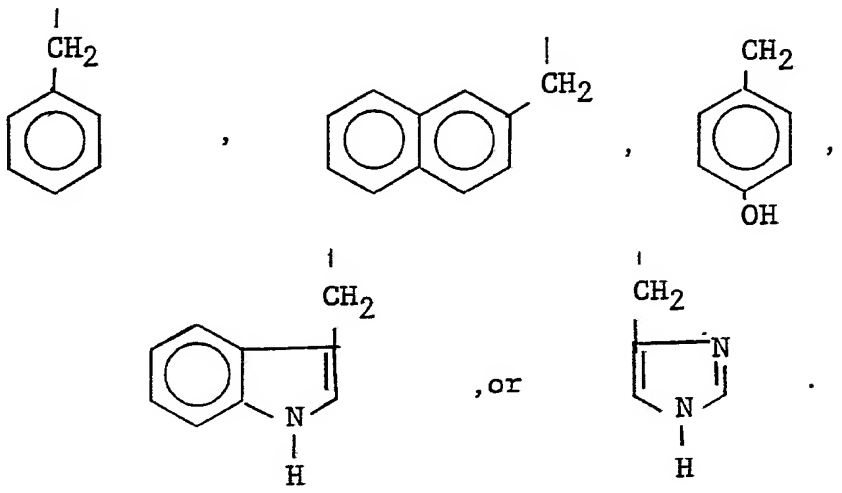
39. The contrast agent of Claim 1 wherein M is selected from iron (II), iron (III) or gadolinium (III), Q and Y are each $-CH_2(C=O)NHCH(R)-(COOR^1)$ wherein R is benzyl and R^1 in Q and Y are each hydrogen, or R^1 in Q is H and R^1 in Y is ethyl, or R^1 in Q and Y are each ethyl, and

J, X, X′ and Z are each $-CH_2(C=O)OH$.

40. The contrast agent of Claim 1 wherein M is selected from iron (II), iron (III), or gadolinium (III), Q and Y are each $-CH_2(C=O)NHCH(R)-COOR^1$, wherein R is phenyl, and R^1 in Q and Y are each hydrogen, or R^1 in Q is H and R^1 in Y is ethyl or R^1 in Q and Y are each ethyl and J, X, X′ and Z are each $-CH_2(C=O)OH$, and m is 1 and n is 0.

41. The contrast agent of claim 1 where m is 1 and n is 0.

42. The contrast agent of Claim 1 wherein R is independently selected from:



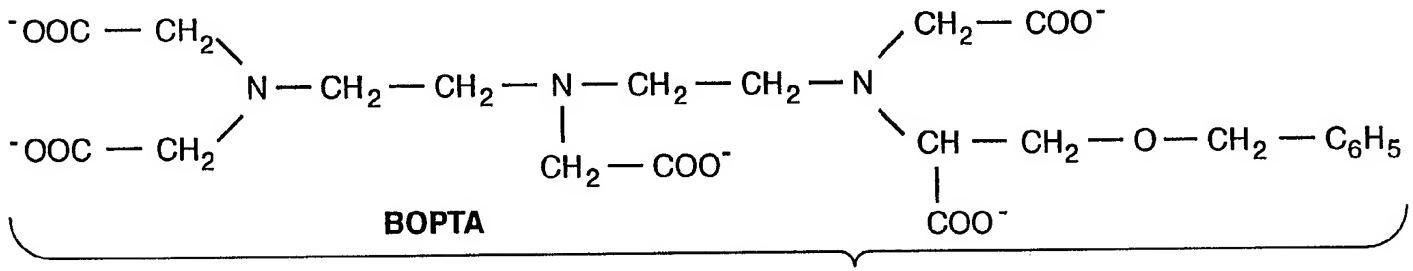


FIG._1A
(PRIOR ART)

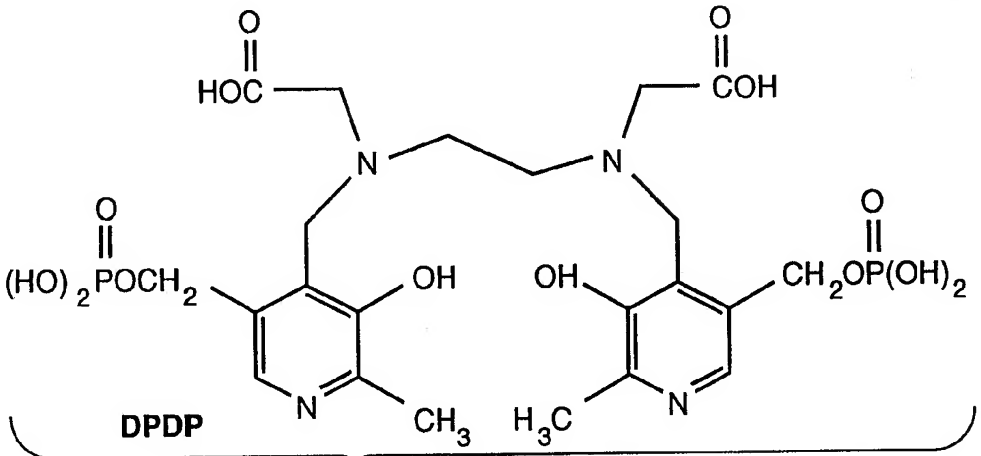


FIG._1B
(PRIOR ART)

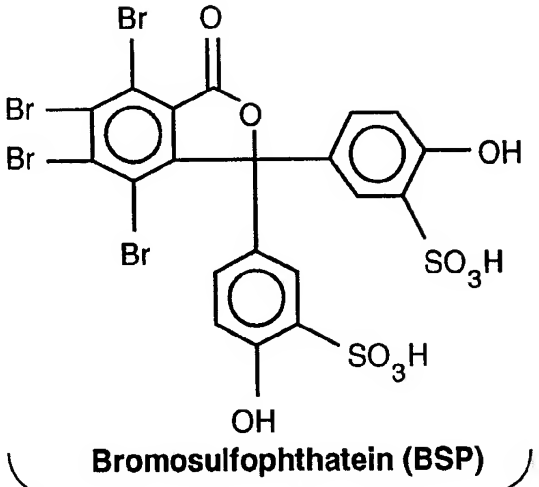


FIG._1C
(PRIOR ART)

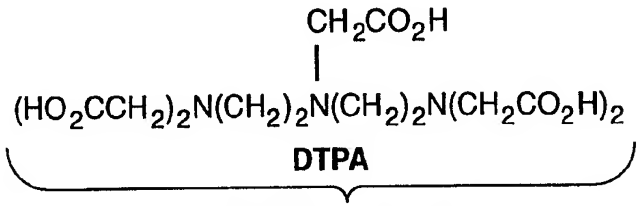


FIG._1D
(PRIOR ART)

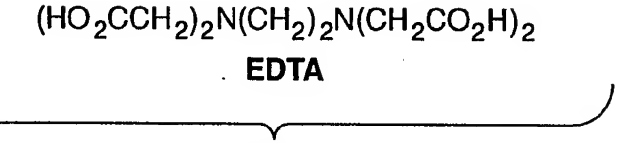


FIG._2A
(PRIOR ART)

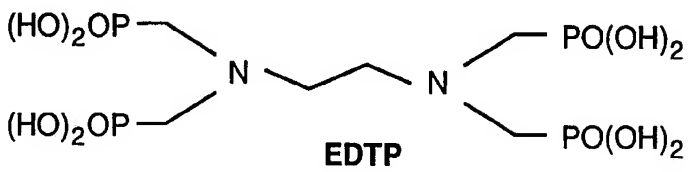
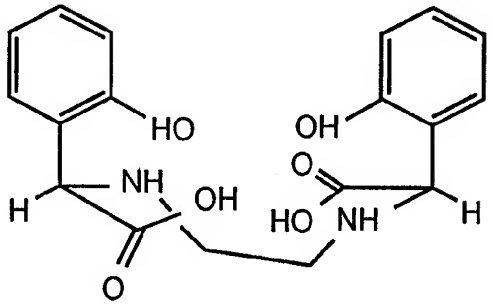


FIG._2B
(PRIOR ART)



N.N'-ethylene *bis* (*o* -hydroxyphenylglycine)

FIG._2C
(PRIOR ART)

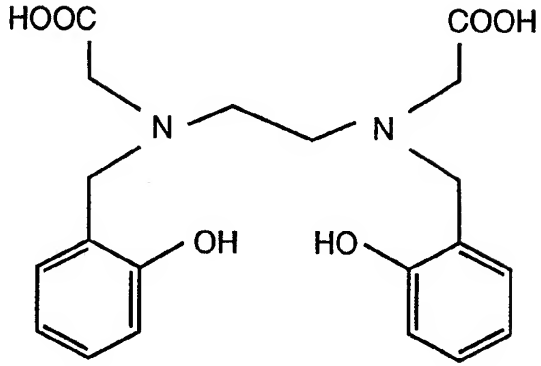


FIG._2D
(PRIOR ART)

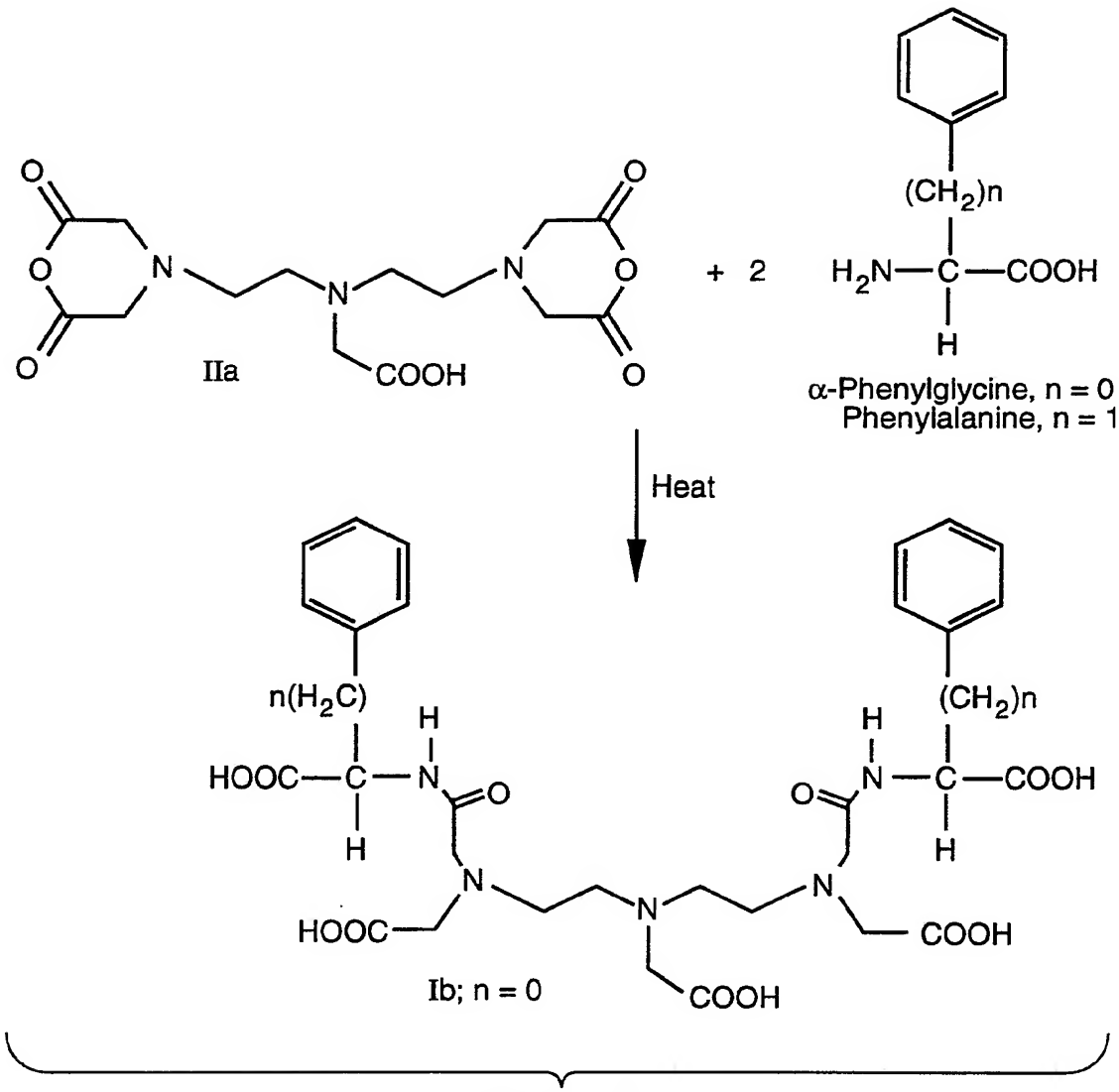

IIa
COOH
+ 2
(CH2)n
H2N—C—COOH
H
α-Phenylglycine, n = 0
Phenylalanine, n = 1
Heat
n(H2C)
HOOC—C—N
(CH2)n
N—C—COOH
HOOC
COOH
COOH
Ib; n = 0

FIG._3

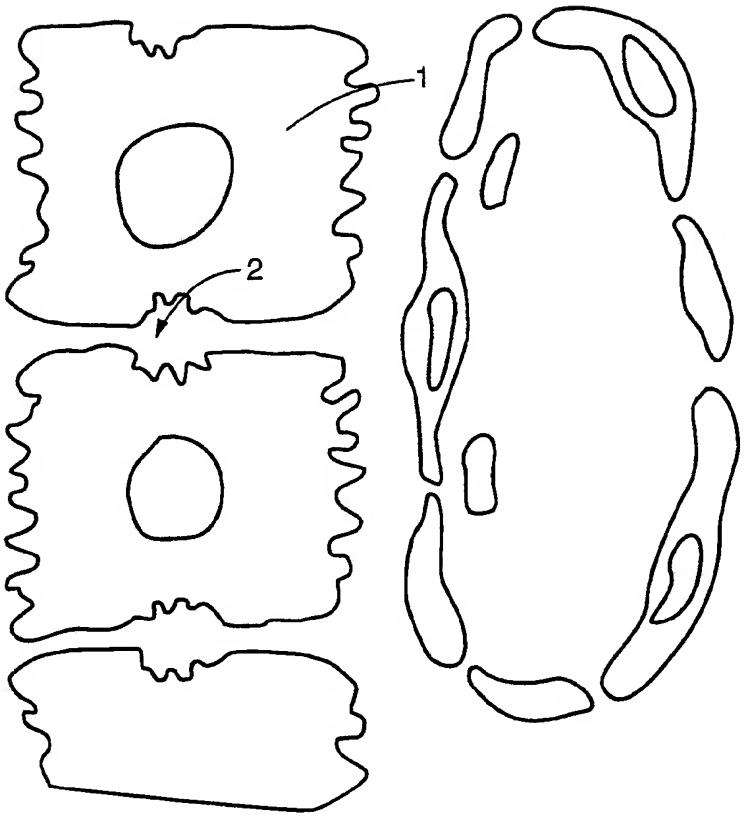


FIG._4

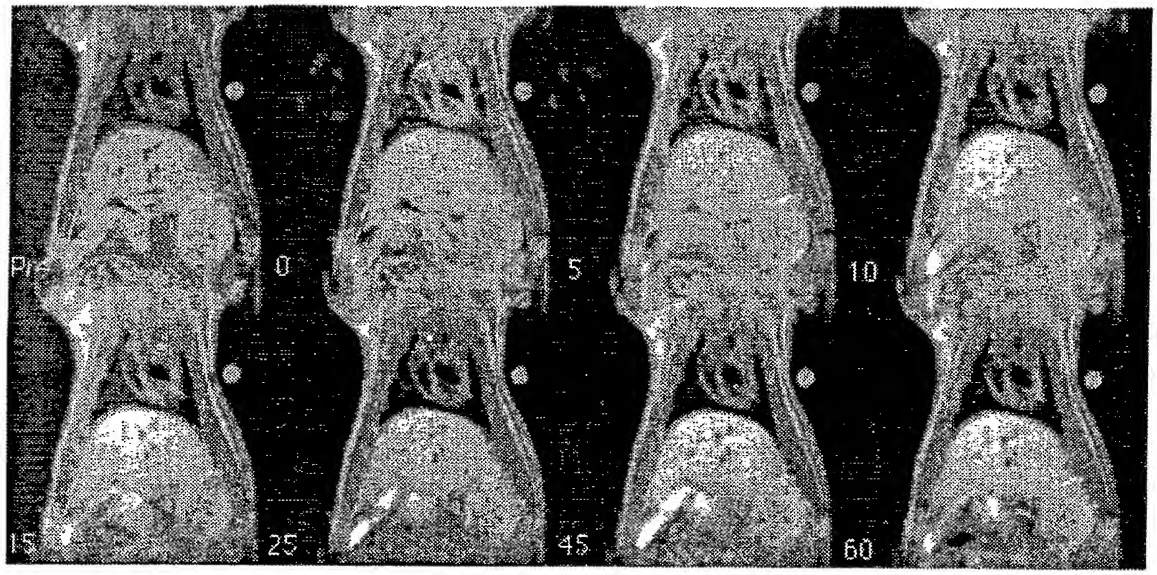


FIG._5A

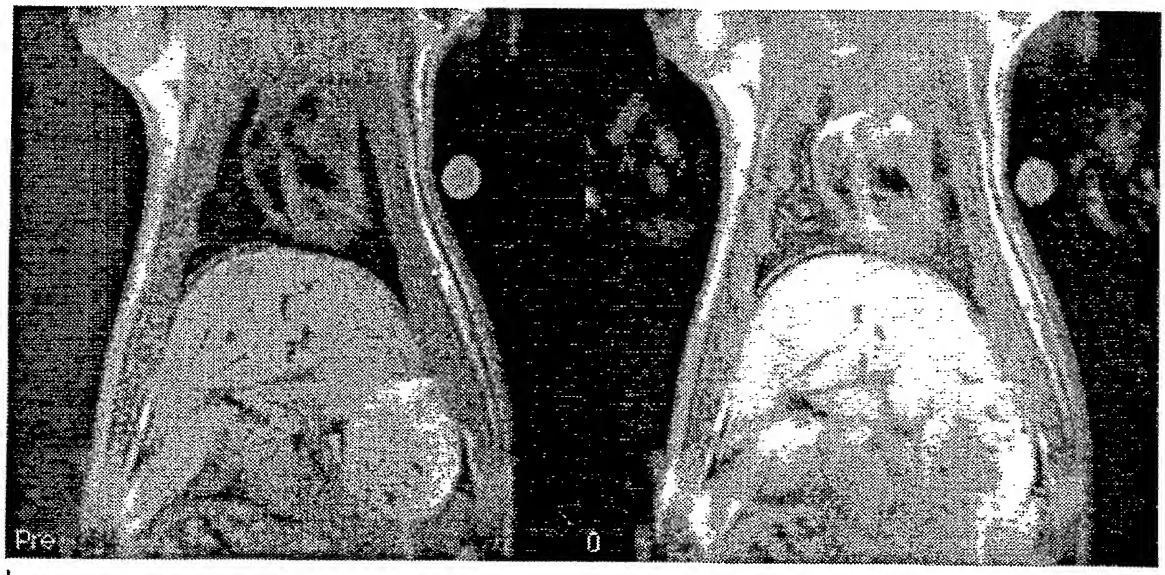


FIG._5B

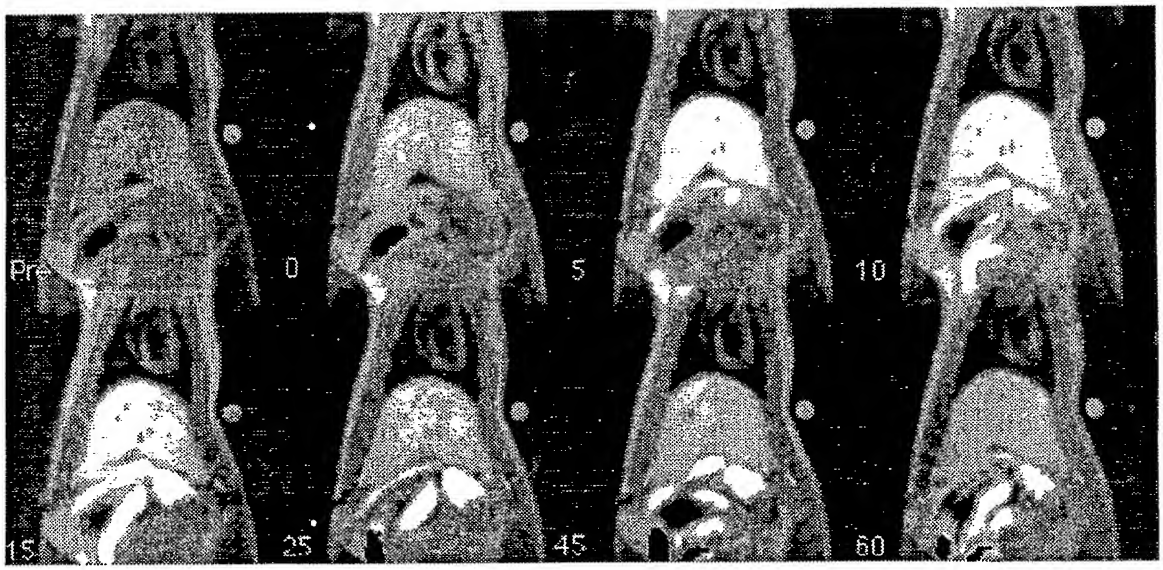


FIG._6A

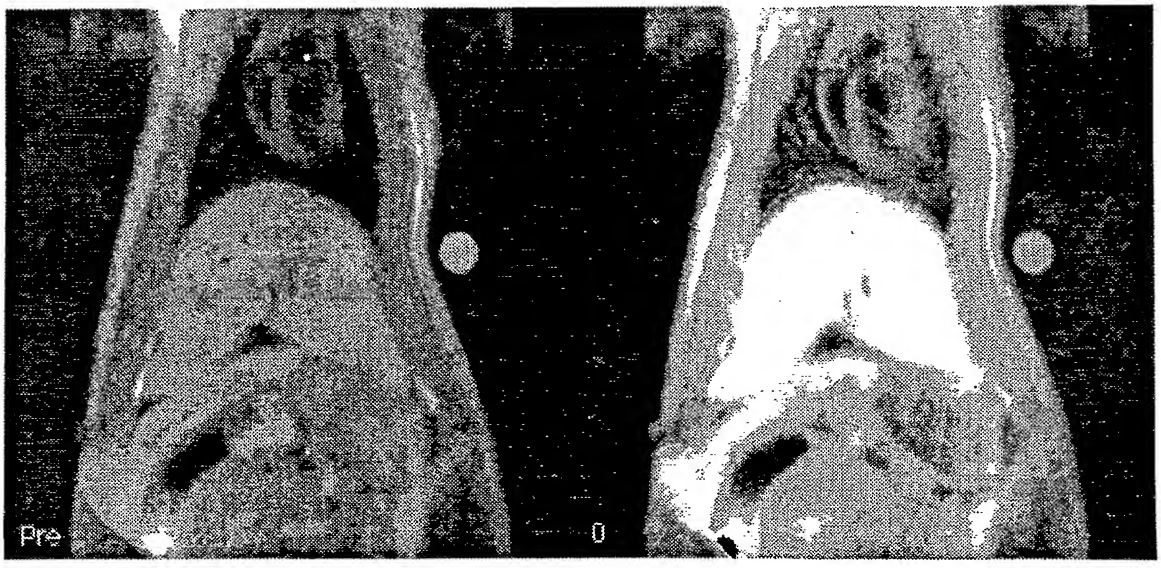


FIG._6B

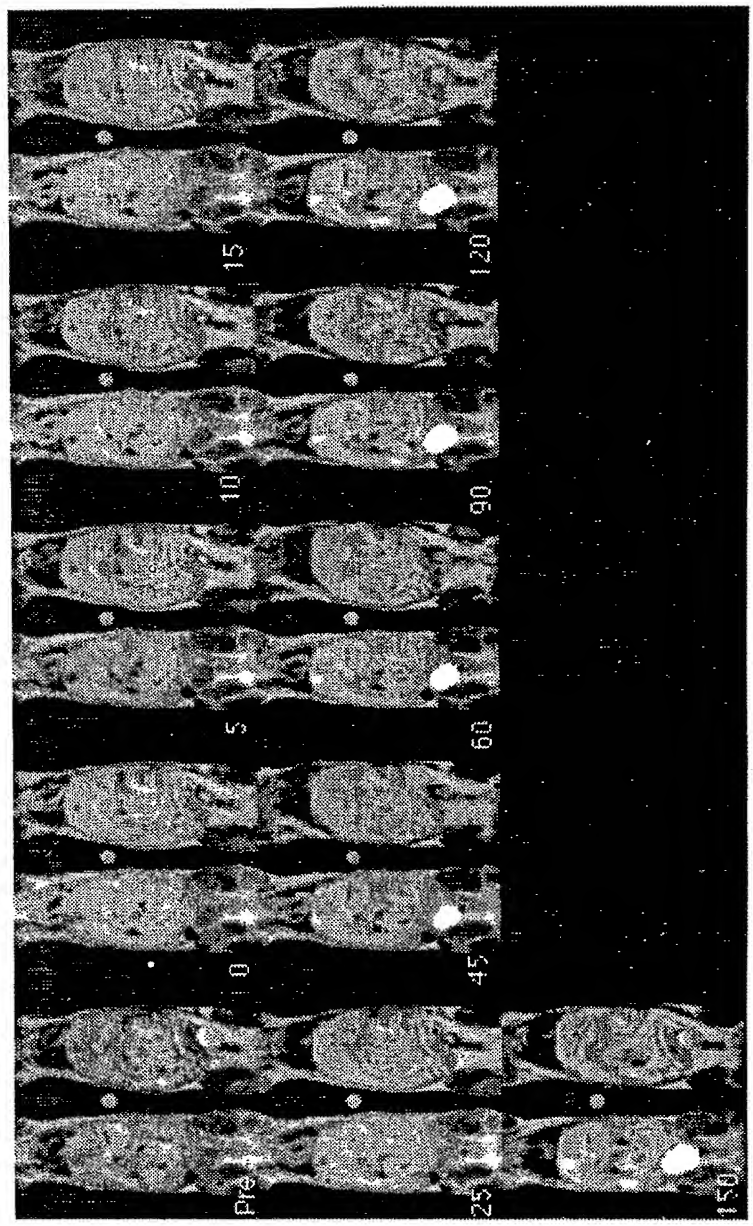


FIG._7

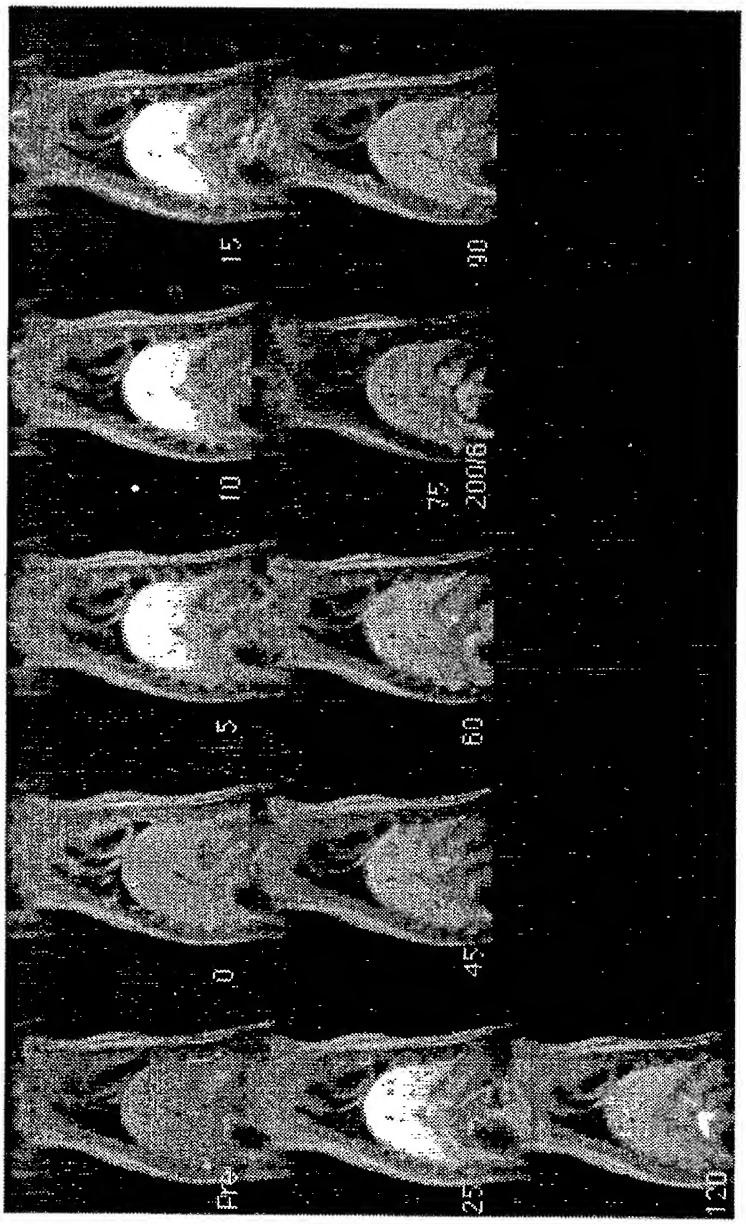


FIG._8

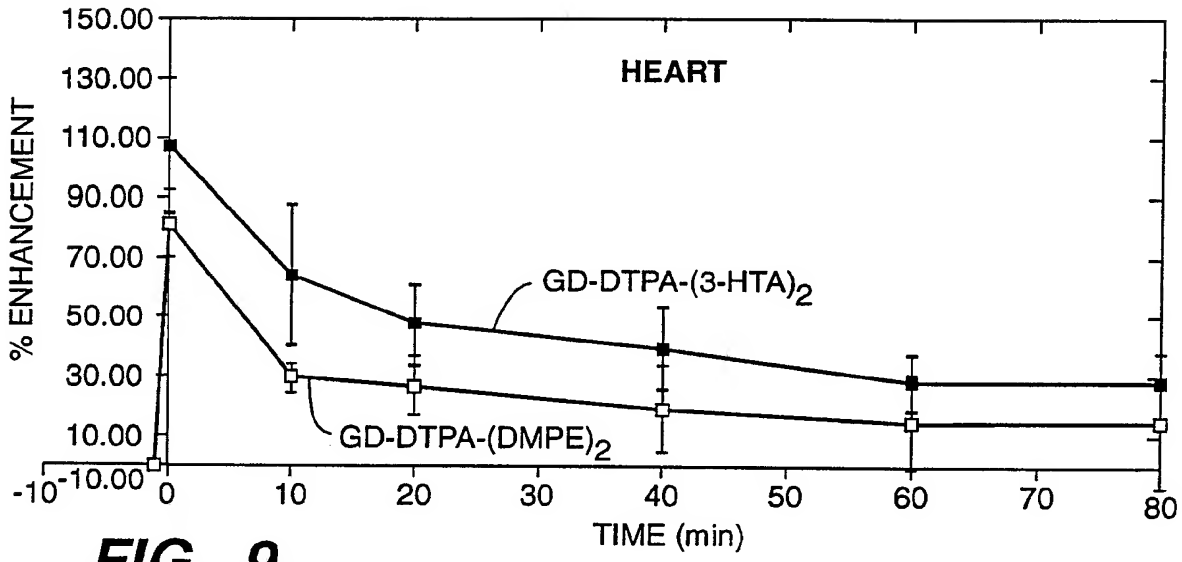

HEART
% ENHANCEMENT
TIME (min)
GD-DTPA-(3-HTA)2
GD-DTPA-(DMPE)2
150.00
130.00
110.00
90.00
70.00
50.00
30.00
10.00
-10.00
-10
0
10
20
30
40
50
60
70
80

FIG._9

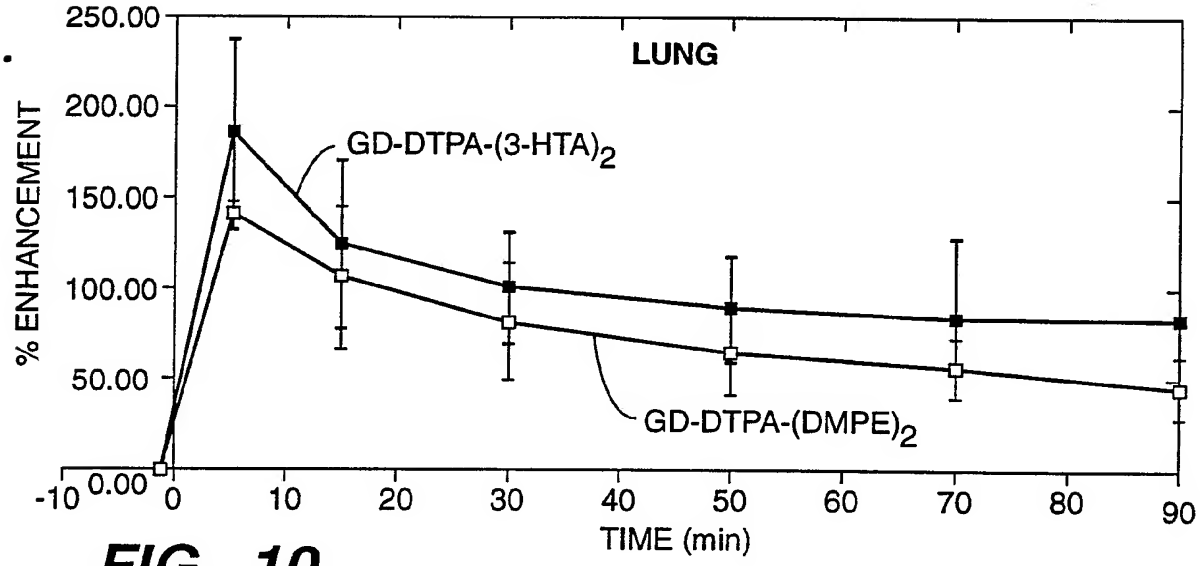

LUNG
% ENHANCEMENT
TIME (min)
GD-DTPA-(3-HTA)2
GD-DTPA-(DMPE)2
250.00
200.00
150.00
100.00
50.00
0.00
-10
0
10
20
30
40
50
60
70
80
90

FIG._10

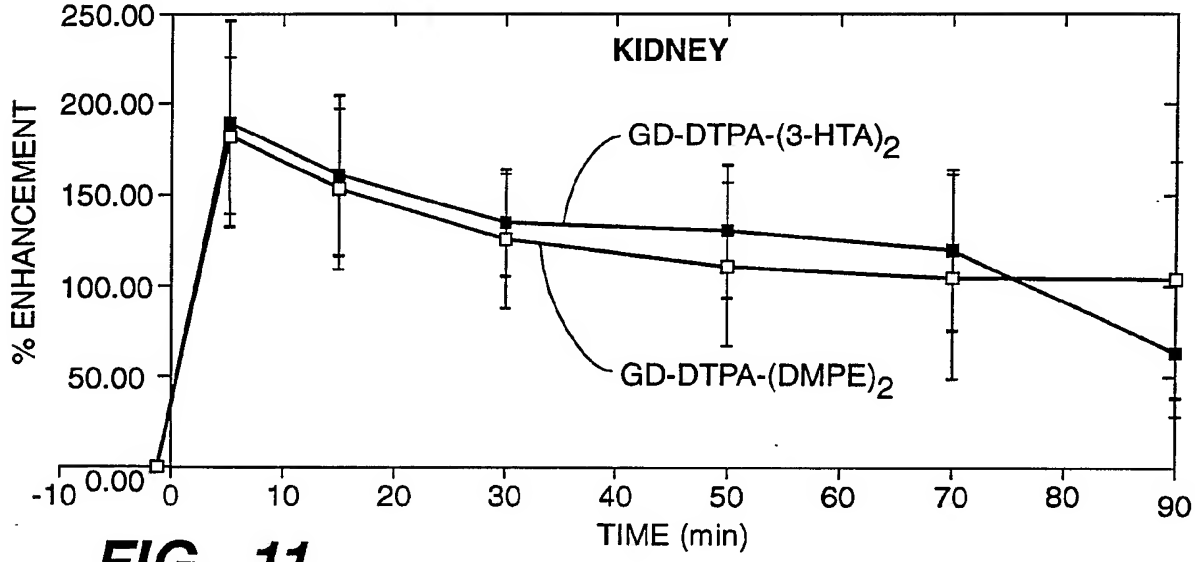

KIDNEY
% ENHANCEMENT
250.00
200.00
150.00
100.00
50.00
0.00
-10
0
10
20
30
40
50
60
70
80
90
TIME (min)
GD-DTPA-(3-HTA)2
GD-DTPA-(DMPE)2

FIG._11

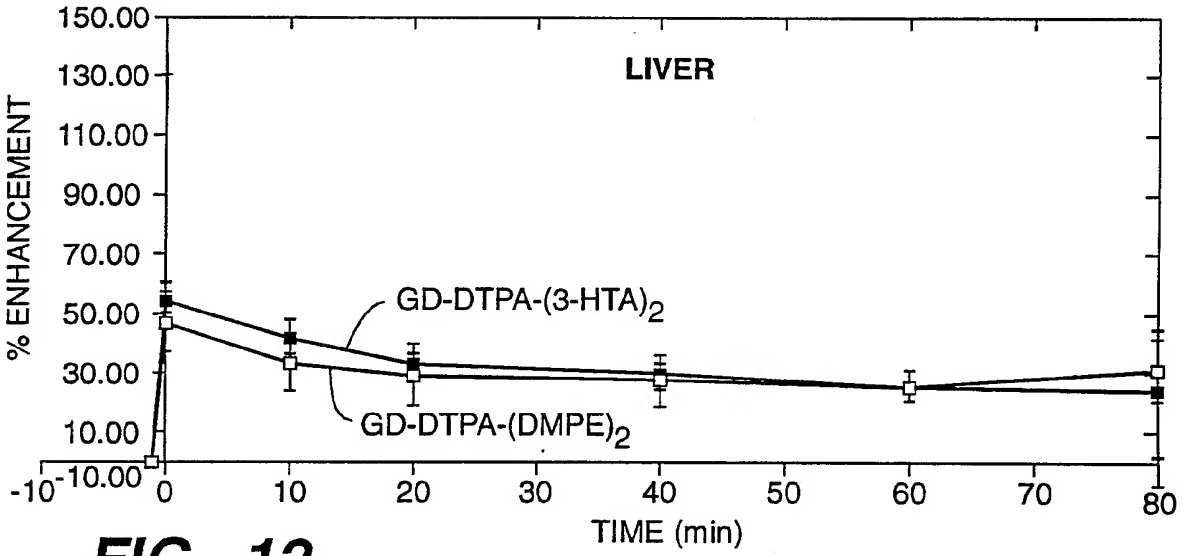

LIVER
% ENHANCEMENT
150.00
130.00
110.00
90.00
70.00
50.00
30.00
10.00
-10.00
-10
0
10
20
30
40
50
60
70
80
TIME (min)
GD-DTPA-(3-HTA)2
GD-DTPA-(DMPE)2

FIG._12

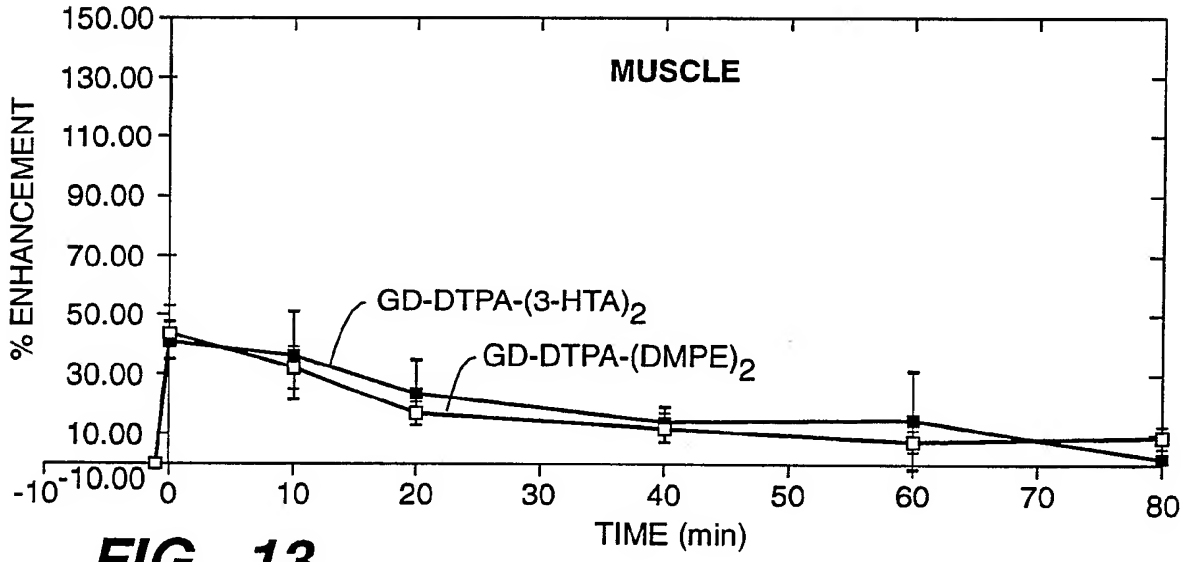

MUSCLE
% ENHANCEMENT
150.00
130.00
110.00
90.00
70.00
50.00
30.00
10.00
-10.00
-10
0
10
20
30
40
50
60
70
80
TIME (min)
GD-DTPA-(3-HTA)2
GD-DTPA-(DMPE)2

FIG._13

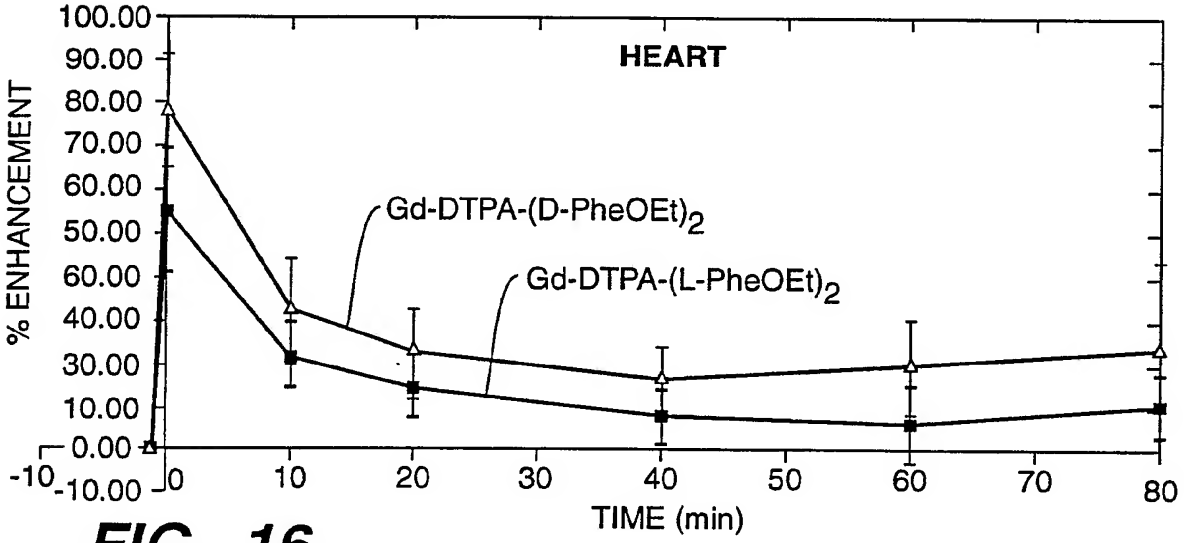

HEART
% ENHANCEMENT
100.00
90.00
80.00
70.00
60.00
50.00
60.00
40.00
30.00
10.00
0.00
-10.00
-10
0
10
20
30
40
50
60
70
80
TIME (min)
Gd-DTPA-(D-PheOEt)2
Gd-DTPA-(L-PheOEt)2

FIG._16

FIG._14A

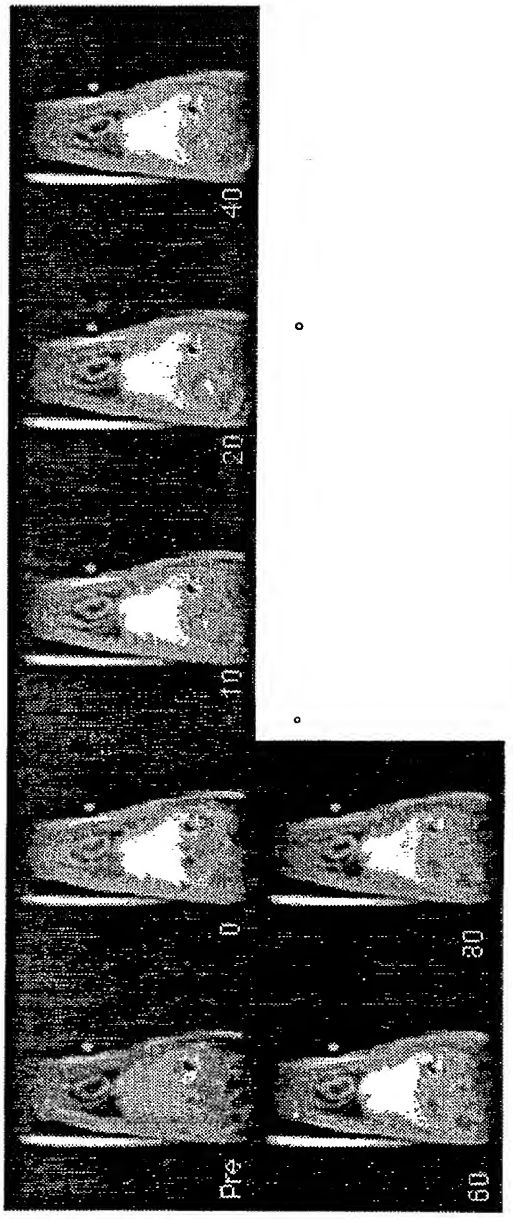


FIG._14B

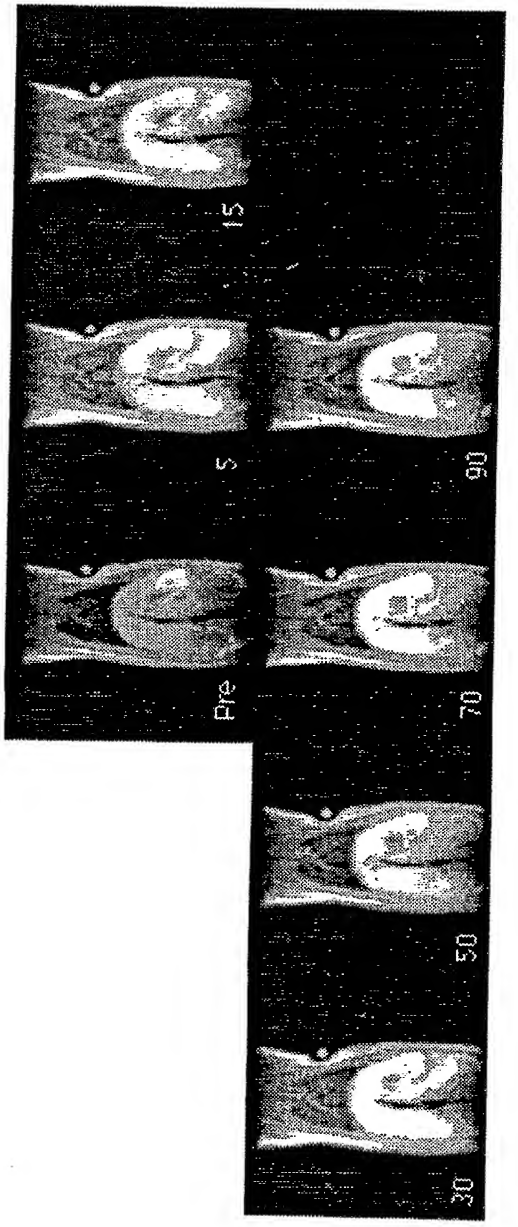


FIG._15A

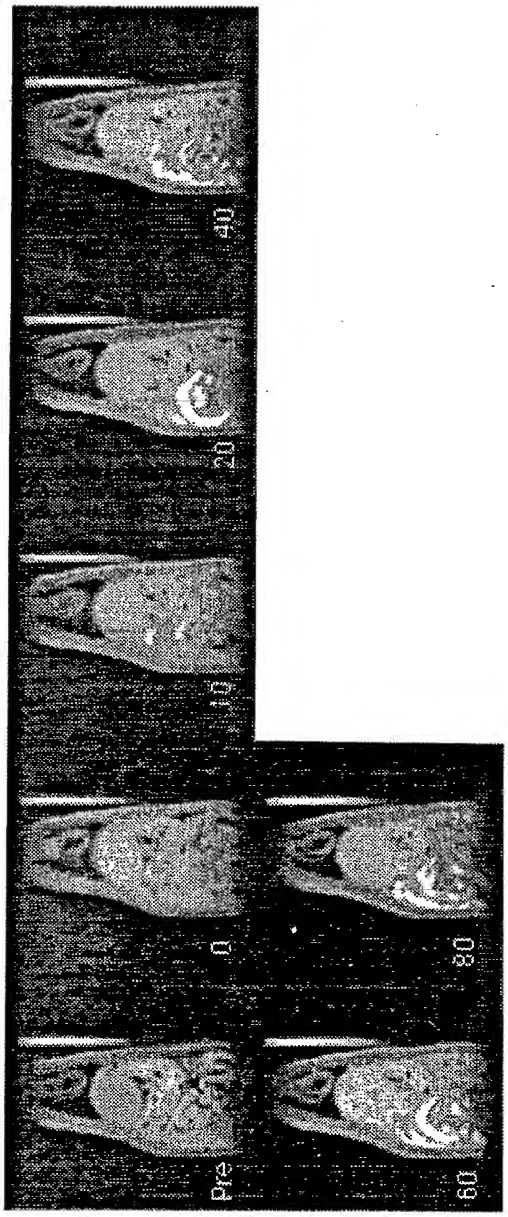
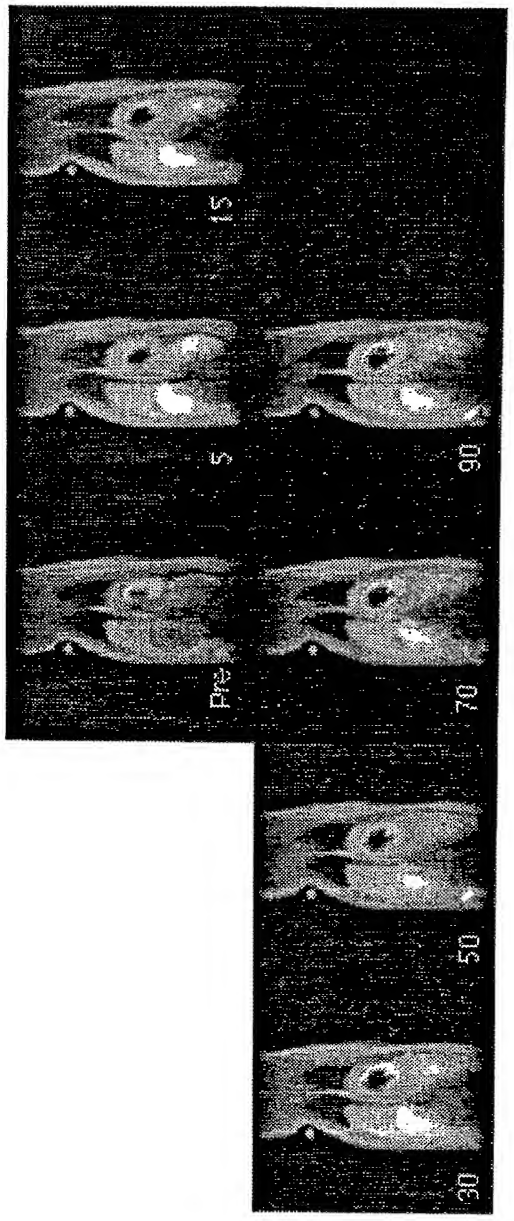


FIG._15B



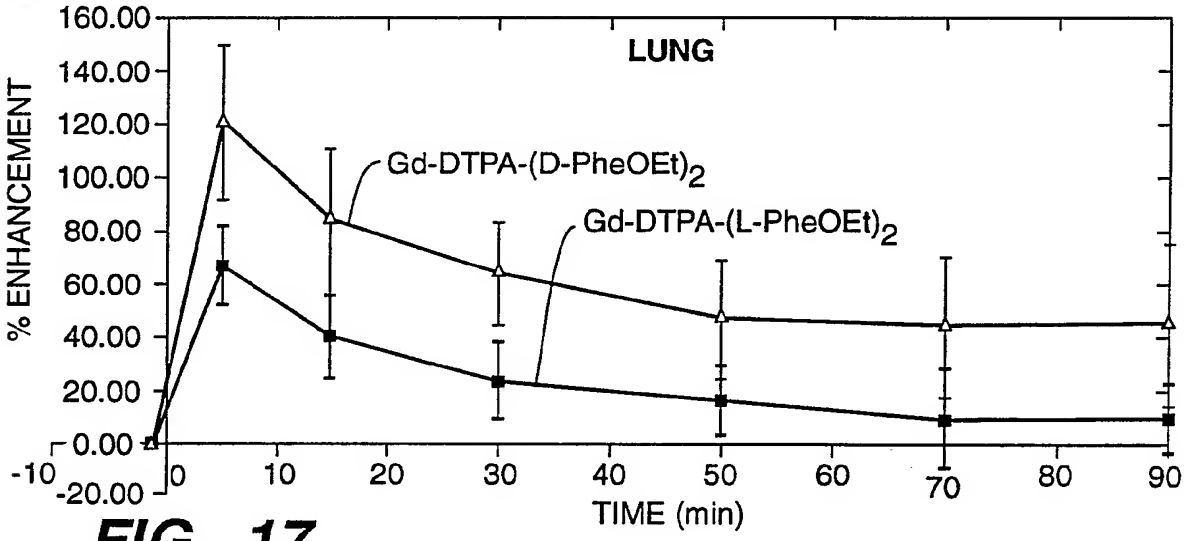

LUNG
% ENHANCEMENT
TIME (min)
Gd-DTPA-(D-PheOEt)2
Gd-DTPA-(L-PheOEt)2
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
-20.00
-10
0
10
20
30
40
50
60
70
80
90

FIG._17

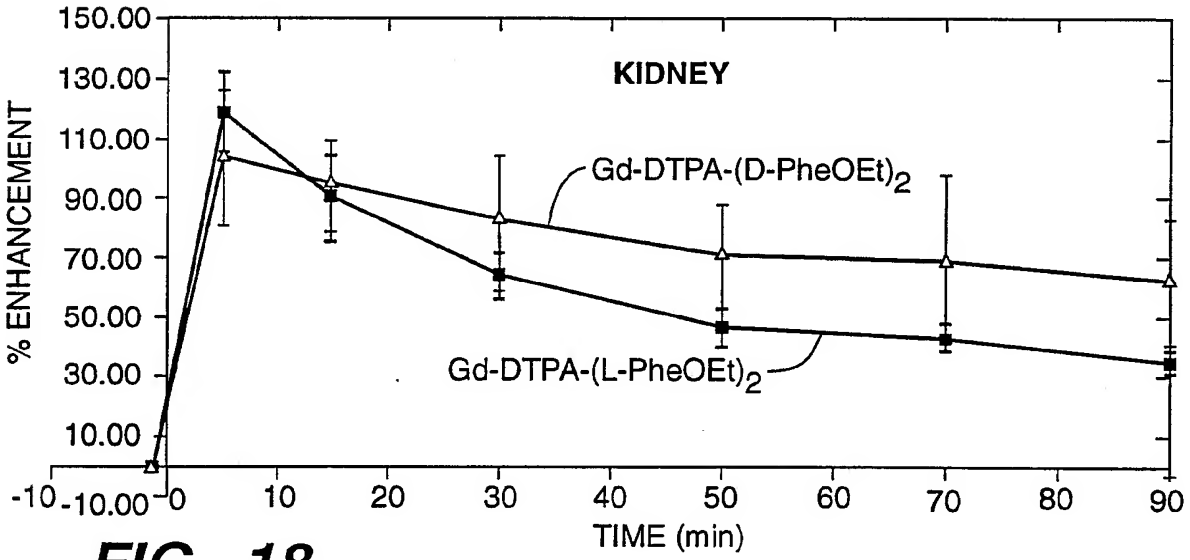

KIDNEY
% ENHANCEMENT
TIME (min)
Gd-DTPA-(D-PheOEt)2
Gd-DTPA-(L-PheOEt)2
150.00
130.00
110.00
90.00
70.00
50.00
30.00
10.00
-10.00
-10
0
10
20
30
40
50
60
70
80
90

FIG._18

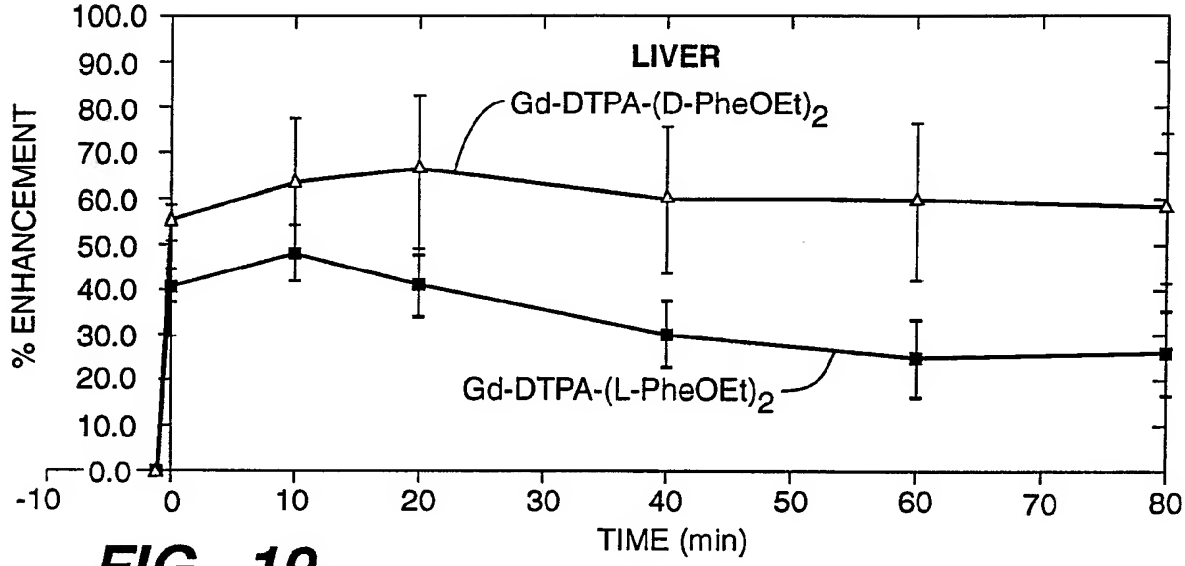

LIVER
Gd-DTPA-(D-PheOEt)2
Gd-DTPA-(L-PheOEt)2
% ENHANCEMENT
TIME (min)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
-10
0
10
20
30
40
50
60
70
80

FIG._19

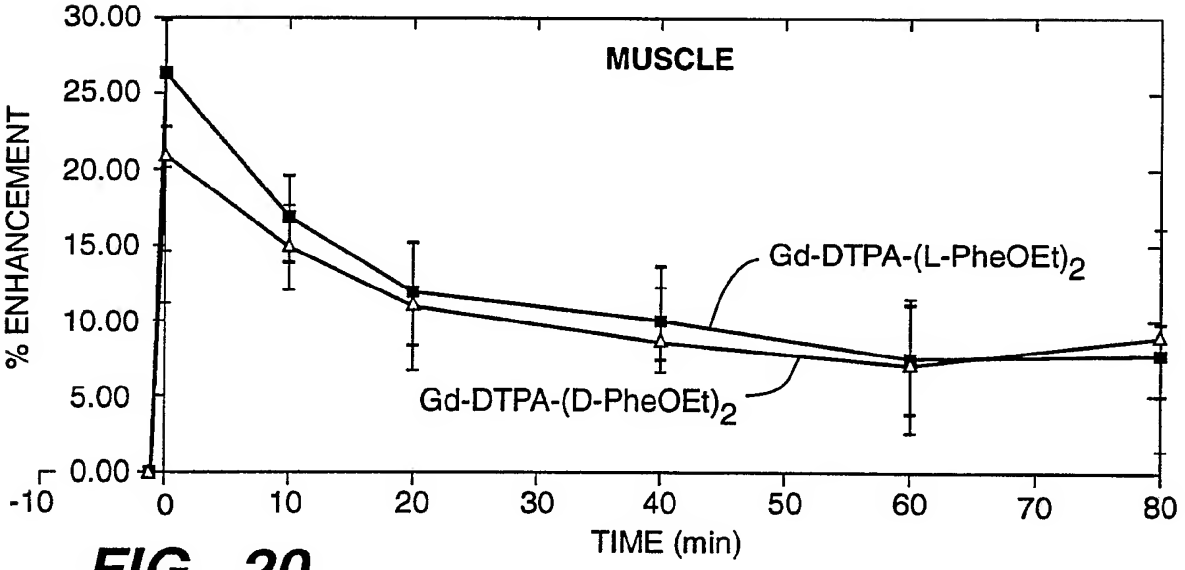

MUSCLE
Gd-DTPA-(L-PheOEt)2
Gd-DTPA-(D-PheOEt)2
% ENHANCEMENT
TIME (min)
30.00
25.00
20.00
15.00
10.00
5.00
0.00
-10
0
10
20
30
40
50
60
70
80

FIG._20

FIG._21A

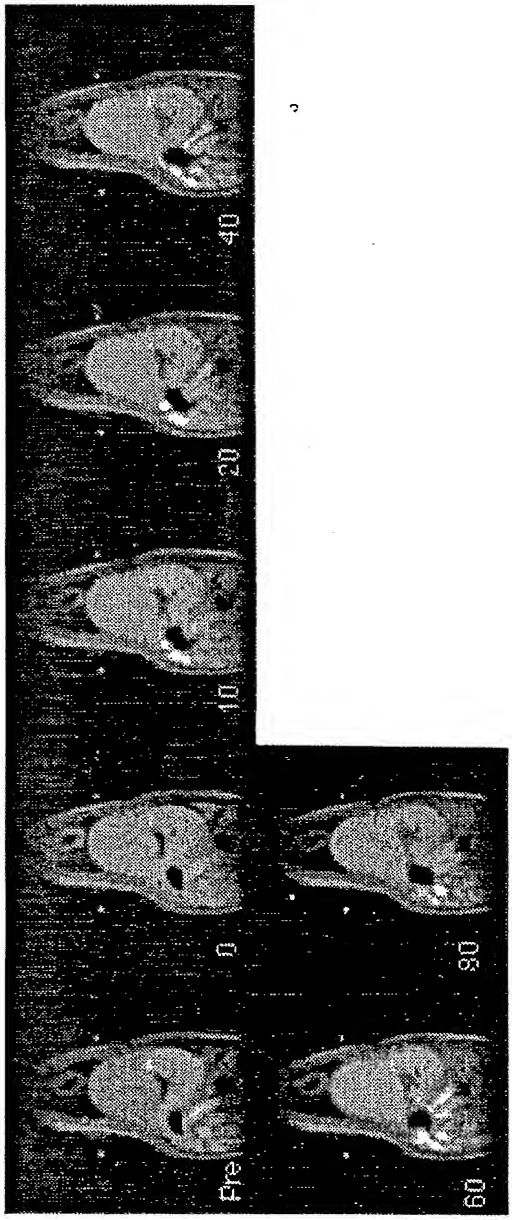


FIG._21B

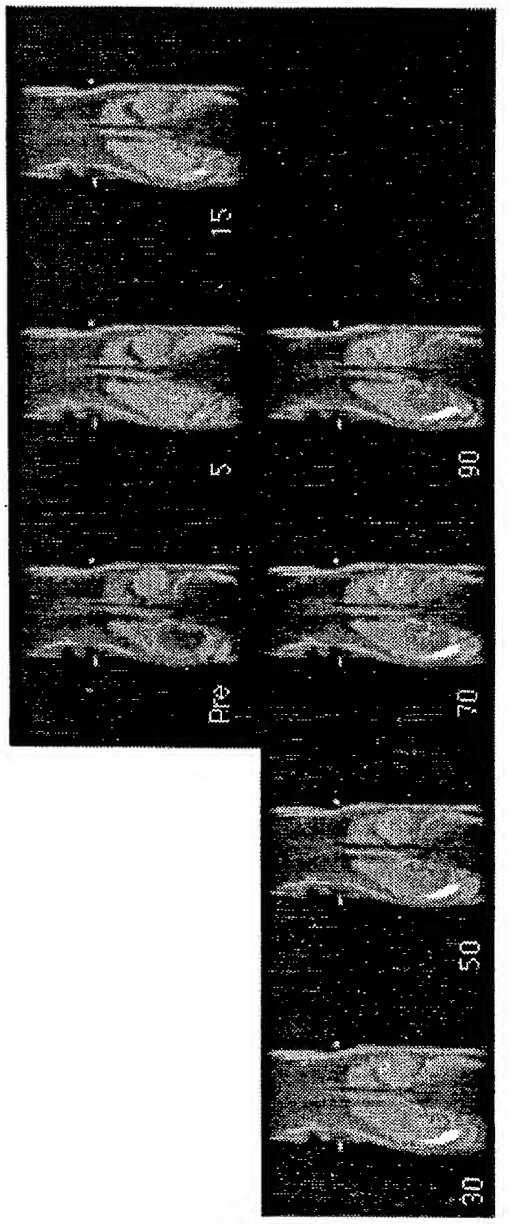


FIG._22A

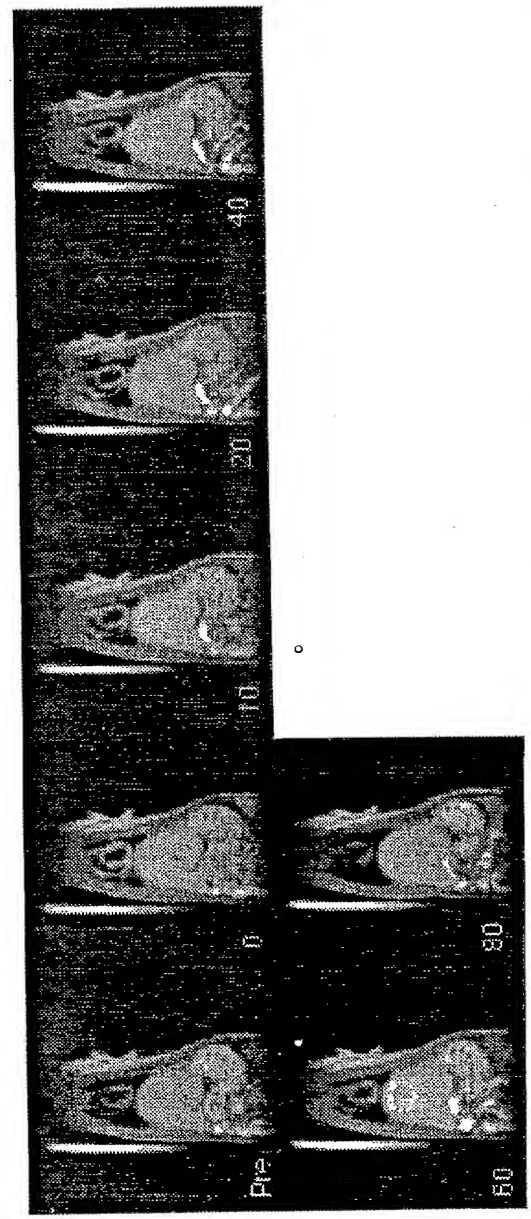
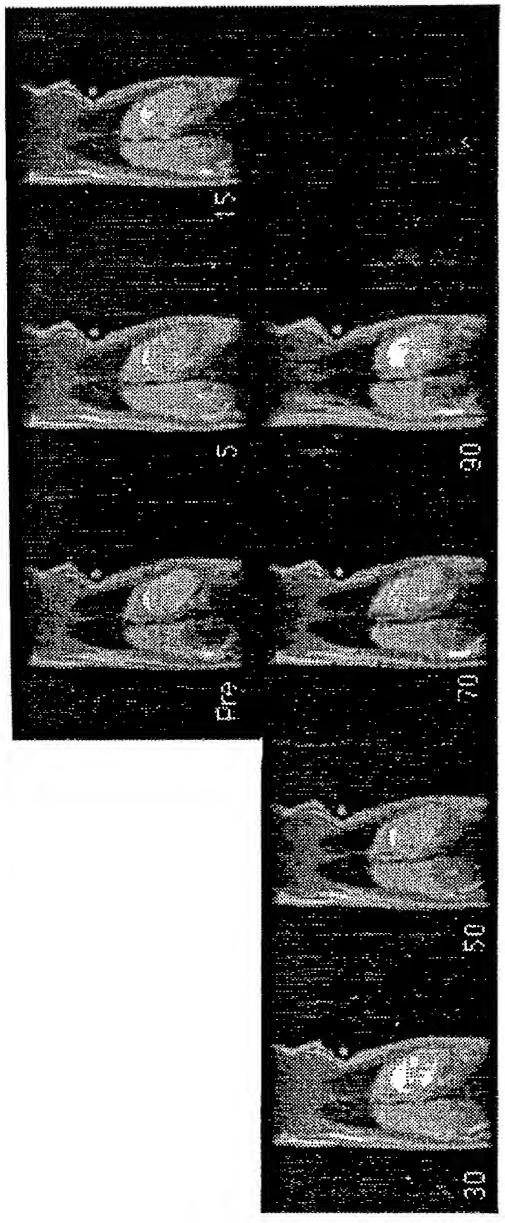


FIG._22B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06660

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 24/08; C07F 19/00
US CL :424/9; 562/565; 436/173,806; 128/653.4,654; 534/16; 544/225; 546/2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,352,751 (WIEDER ET AL.) 05 October 1982, See abstract and col. 8, line 48 - col. 9, line 48.	1-21
Y	US, A, 2,394,230 (BILLMAN) 05 February 1946, See formula 14.	1-21
Y	WO, A1, 89/06979 (KLAVENESS ET AL.) 10 August 1989, See claim 1 and example 1.	1-21
Y	WO, A1, 91/05762 (LOVE, ET AL.) 02 May 1991, See claim 1.	1-21
Y	FR, A, 2,045,987 (STAUFFER CHEMICAL COMPANY) 05 March 1971, See pages 4-8.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 21 OCTOBER 1992	Date of mailing of the international search report 10 DEC 1992
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer for GARY E. HOLLINDEN NGUYEN NGOC-HO INTERNATIONAL DIVISION Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06660

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Lack of Unity is apparent in the claimed inventions as identified herein:

I. The contrast agent of formula Ia wherein at least one R is a heteroaromatic group (amino acid), a process of preparing said compound and a method of using said compound for magnetic resonance imaging.

II. The contrast agent of formula wherein all of the R groups are hydrogen, aromatic or alkyl (amino acid), a process of preparing said compound and a method of using said compounds for MRI.

Claims 1-21 are generic to both of the grouped inventions.

Clearly, a reference which would anticipate Group I would not necessarily anticipate or even make obvious the invention(s) of Group II. Further, the searches of the inventions are not co-extensive, particularly with regard to the literature search required and would constitute an undue burden for the Examiner. Since the compounds as grouped represent independent classes of compounds, each is capable of supporting its own patent.

Because these inventions do not provide a single general inventive concept for the reasons given above, restriction for examination purposes as indicated is proper.